THE EARLY DIAGNOSIS OF NEONATAL SEPSIS

An evaluation of leucocyte counts and acute phase reactants

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APPENDIX

PUBLICATIONS

1) Acta Paediatr. Scand. 1979, 68:481-483

2) Pediatrics 1980, 65:1036-1041

3) J. Pediatr. 1981, 98:795-799

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ABSTRACT OF THESIS (Regulation 7.9)

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Title of Thesis THE EARLY DIAGNOSIS OF NEONATAL SEPSIS: An evaluation of leucocyte counts and acute phase reactants.

Several laboratory tests suggested as being helpful in the diagnosis of neonatal sepsis were evaluated individually and in combination. The tests chosen were considered to be rapid, simple and inexpensive, so that they could be generally available.

There were 524 babies evaluated for sepsis in the first week after birth and 56 babies evaluated between 8 and 60 days of age. The reasons for investigation were varied, being primarily "risk factors" (e.g., prolonged rupture of membranes, maternal fever, etc.) in the first two days and "clinical factors" (e.g., apnoea, lethargy, abdominal distension, etc.) beyond that time.

Of the babies investigated during the first week, 41 proved to have sepsis and in another 34 it was "very probable" that infection was present. Among the older infants, 12 had sepsis, 2 had necrotising enterocolitis and 5 had "very probable" infection.

No single test can be considered entirely satisfactory, but the single most useful test seems to be the immature/total neutrophil ratio. Unlike some other tests, it does not seem to be influenced by birth weight or gestational age. When the immature/total neutrophil ratio (or fraction) was ≥ 0.2, 34/41 infants with sepsis were detected in the first week (sensitivity = 83%), and 7/12 (58%) between 8 and 60 days, but positive predictive accuracy (number of proved cases with a positive test/total positive tests) was only 24% and 40% respectively. In contrast, a combination of tests designated as a "sepsis screen" (two or more of five diagnostic findings: wbc $< 5.0 \times 10^9/\ell$, I/T ratio ≥ 0.2, ESR ≥ 15 mm/h, latex C-reactive protein positive, and latex haptoglobin positive) detected 38/41 infants with sepsis in the first week (sensitivity = 93%) and 10/12 (83%) between 8 and 60 days. Positive predictive accuracy for the sepsis screen was 38% (0-7 days) and 43% (8-60 days). When "infection" (sepsis plus "very probable") was evaluated, sensitivity of the sepsis screen was 89% and positive predictive accuracy was 68% for infants aged 0-7 days. The most useful pair of tests in the first week after birth was wbc $< 5.0 \times 10^9/\ell$ and I/T ratio ≥ 0.2 which detected 15/41 infants with sepsis (37%) with a positive predictive accuracy of 68% for sepsis and 86% for "infection." Between 8 and 60 days, the best pair of tests was latex CRP positive and I/T ratio ≥ 0.2 with 6/12 (50%) infants with sepsis detected and positive predictive accuracy of 60% for sepsis and 80% for "infection."

In the second half of the study, the tests were used at the bedside. Antibiotic use decreased in those babies who subsequently proved not to be infected. In the first half of the study, only 14% of the babies evaluated did not receive antibiotics, but in the second half of the study, this number increased to 47%. This reduced the ratio of babies treated with antibiotics to babies with proven sepsis from 11:1 to 6.6:1.

Although these tests are non-specific indicators of infection, they are sufficiently simple and inexpensive to be used in almost any hospital where babies are delivered. There is some evidence (which needs to be confirmed) that acute phase proteins are valuable, not only in diagnosis, but also in following the course of the illness. This should allow more rational decisions to be made both in starting and in stopping antibiotics.

INTRODUCTION

> "Diagnosis of neonatal septicemia is one of the most difficult tasks in clinical medicine."
> (Quie, 1976[1])

> "... theoretically all cases of neonatal septicemia may be treated successfully. Thus, there is an urgent need for early diagnosis and proper management."
> (Gotoff and Behrman, 1970[2])

The difficulty in making a diagnosis of neonatal sepsis (septicaemia) can be attributed to the wide variety of clinical manifestations which may be indicative of this disease.[1-5] Neonatal sepsis may be defined as a systemic disease of the newborn infant, associated with the presence in the blood of pathogenic micro-organisms (usually documented by a positive blood culture). Neonatal meningitis is frequently indistinguishable from sepsis on clinical grounds and may occur concomitantly. Thus, although we usually talk about making a diagnosis of sepsis (as I shall do), it should be remembered that this is more correctly stated as making a diagnosis of sepsis or meningitis.

Despite many advances in neonatology, neonatal sepsis remains a major cause of mortality and morbidity, particularly in the preterm infant.[5,6] Indeed, as a result of significant decreases in other causes of neonatal mortality, neonatal sepsis has assumed a more prominent role in mortality in recent years. For example, at the Medical Center Hospital of Vermont (where this study was conducted), the number of neonatal deaths attributable to bacterial infection, in the inborn population of almost 10,000 babies in the years 1975 through 1979, was 11 out of a total of 103 neonatal deaths. When

deaths attributable to birth weight less than 1000 grams (n = 46), congenital abnormalities incompatible with life (n = 26) and hydrops foetalis (n = 5) were excluded, the cause of death in 8 out of the remaining 26 babies (31%) was sepsis.* In some African countries (e.g., Ethiopia) neonatal sepsis may account for an even higher percentage of neonatal deaths. Bacteraemia was present in 140 of 260 babies (54%) who died, and in 70% of babies greater than 35 weeks gestation who died.(7)

It is apparent that neonatal sepsis remains a significant problem throughout the world and that constant vigilance is required to diagnose and treat it early. Because of the association of sepsis with certain risk factors (e.g., maternal fever, prolonged rupture of membranes, etc.) and a multiplicity of clinical signs (e.g., lethargy, temperature instability, apnoea, etc.), the pendulum may have swung too far regarding treatment. It seems fair to say that, 30 years ago, paediatricians were not as aware of the subtle manifestations of neonatal sepsis as they are today, so that babies went untreated.(8) Currently, the situation seems to be reversed; paediatricians recognise that many infants are at risk and institute investigations only briefly before moving to antibiotic therapy. This policy may result in many babies being treated unnecessarily.(5,9) Because the indiscriminate use of antibiotics may lead to the emergence of resistant organisms,(10,11) it seems important to be able to diagnose infection more reliably, in order to use antibiotics more discriminantly.

* - personal unpublished observations

Although it is agreed that the most important part of diagnosis is clinical suspicion, the non-specificity of clinical manifestations in the neonate[1-5] (particularly those with respiratory distress[12-14]) dictates that some adjunctive information be used to distinguish the infected from the non-infected infant. A variety of laboratory tests have been proposed as helpful in making a diagnosis of sepsis (or infection). During the past decade or so, a considerable amount of information has been gathered, in an attempt to define a single test which could be used as a reliable diagnostic indicator (sometimes called an indicant[15]). In some cases, assessment has been made retrospectively, by looking at the findings in infants with documented sepsis and then choosing a control population with which to compare them. It seems important to evaluate infants suspected of having sepsis prospectively, to decide which pieces of information might be helpful at a time when the clinician is faced with the decision of whether or not to treat with antibiotics.

The indicant (diagnostic test) which has attracted most attention is the leucocyte count and differential count.[6,13,14,16-29] Some authors have concentrated on total leucocyte or total neutrophil counts,[6,13,17-19] while others have been more interested in the total immature neutrophil count[16,20,21] and (most recently) the immature/total neutrophil ratio (fraction)[23,25,28] or immature/mature neutrophil ratio (fraction).[16,24] The other indicants proposed include: platelet count,[30,31] erythrocyte sedimentation rate,[32,33] gastric aspirate smear,[34-36] buffy-coat smear,[13,37] nitro-blue tetrazolium reduction test,[38-40] umbilical cord

histology,[41-43] immunoglobulin M[44,45] and a number of acute phase proteins, such as haptoglobin,[46] C-reactive protein,[47,48] α_1-acid glycoprotein (orosomucoid)[49-51] and fibrinogen.[52-54]

The purpose of the studies presented here was to evaluate infants suspected of having sepsis, using several laboratory tests suggested as helpful in diagnosing neonatal infection, to determine which test or combination of tests was most helpful in confirming that neonatal sepsis was present. The basis for choosing the tests was that they could provide a rapid result (preferably within an hour or less) and that they were simple and relatively inexpensive. Such tests could presumably be available (or applicable) in any hospital in the world.

Many of the findings of these studies have been published previously and may be found in the Appendix.

SUBJECTS

> "The wide range of signs and symptoms which may characterize the clinical syndrome of septicemia often suggests a variety of alternative diagnoses ..."
>
> (Gotoff and Behrman, 1970[2])

> "The clinical manifestations of neonatal sepsis are varied and often quite subtle. To compound the clinician's dilemma, many signs overlap with those of conditions of very different etiology ..."
>
> (Daum and Smith, 1979[4])

All infants admitted to the Intensive Care Nursery of the Medical Center Hospital of Vermont between October 1975 and March 1980 were eligible for entry into the study. Any baby at risk for, or suspected on clinical grounds of having, sepsis or meningitis, who had a "sepsis work-up" including a blood culture, was included in the study. The housestaff were also provided with the following "risk" or "clinical" factors, which might suggest that such a "sepsis work-up" should be done. However, not all babies with the factors listed were evaluated with blood cultures.

Risk Factors:

1. Prolonged rupture of membranes (> 24 hours)
2. Prolonged labour (> 24 hours)
3. Maternal fever or other evidence of maternal infection
4. Foul-smelling amniotic fluid or baby
5. Preterm labour without satisfactory explanation
6. Small-for-gestational age (SGA) baby
7. Meconium-stained amniotic fluid without satisfactory explanation
8. Foetal tachycardia on foetal heart rate monitor

Clinical Factors:

1. Lethargy
2. Poor feeding
3. Abdominal distension
4. Temperature instability after 4 hours of age
5. Unexplained jaundice
6. Unexplained apnoea
7. Unexplained cyanotic spells
8. Petechiae and purpura (not confined to head and shoulders)
9. Irritability
10. Hepatosplenomegaly
11. Diarrhoea
12. Pustules

Some of these items may benefit from a word of explanation. A "satisfactory explanation" for preterm labour would include things such as multiple pregnancy and antepartum haemorrhage, and for meconium-stained amniotic fluid would include post-term delivery and umbilical cord prolapse. Infants who were SGA were included to try to include some infants with non-bacterial infection for comparison.

Temperature instability included hypothermia ($< 36^{o}C$) or fever ($> 37.5^{o}C$) or variability of $1^{o}C$ or more between readings. Unexplained jaundice was usually a bilirubin level higher than expected at any age in the absence of blood group incompatibility or morphological abnormality of the red blood cells. Apnoea and cyanotic spells were "explained" in the presence of intrinsic pulmonary disease, intra-ventricular haemorrhage or metabolic derangement. Clinical items 11

and 12 were included for older infants.

It should also be mentioned that profound shock and pallor, bleeding diathesis, convulsions and sclerema were not listed, because these are late manifestations of sepsis and the intent was to make an early diagnosis.

There were approximately 2000 infants admitted to the Intensive Care Nursery during the period of the study. Of these admissions, 524 babies were evaluated during the first week after birth, and a further 56 babies were investigated after the first week.

Designation of infection status had to be made retrospectively, since all babies were considered at risk for, or demonstrated clinical evidence of, sepsis. Those babies with positive blood and/or CSF and/or urine cultures (within 48 hours of incubation) were considered to have "proven" sepsis. Babies who received antibiotics for 3 days or less, and who survived (or died without evidence of infection at necropsy) were considered to be "not infected." The remainder can be considered uncertain, but were included as not infected in most analyses. However, a number of babies (see Results) had strong presumptive evidence of systemic infection (although blood and CSF cultures were negative). The babies in this group were considered to have "very probable" infection and were included with the proven cases as "infection" in other analyses.

METHODS

> "Clearly we need an infallible test or combination of tests for bacteraemias that is easily performed with results available within a short time."
>
> (Editorial, Br. Med. J., 1979[5])

As indicated earlier, these infants were evaluated prospectively, but the analyses were conducted retrospectively. Each infant who had a "sepsis work-up" had blood sent for aerobic and anaerobic cultures, and most had urine (suprapubic tap) and cerebrospinal fluid sent for culture. A gastric aspirate was sent for smear to detect leucocytes or bacteria in babies evaluated early (when indicated), and a white blood cell (leucocyte) count and differential count were performed on all babies. Platelet estimates were performed on all babies, and when low or equivocal, a platelet count was performed. Samples for viral cultures were sent to a research laboratory within the University in any case where viral infection seemed likely. Chest radiographs were obtained in babies with associated respiratory signs.

In addition to these items, which may be considered as standard practice in the hospital (and elsewhere), an extra blood sample (0.5 to 1.0 ml) was taken for the following studies (after obtaining permission from a parent):

1. Immunoglobulin M (IgM) - performed by gel radial immunodiffusion technique,[55] as well as a rapid latex method[56]
2. C-reactive protein (CRP) - using a rapid latex method[57]

3. Haptoglobin (Hp) - by the Tarukoski method[58] and a rapid latex method[57]
4. Erythrocyte sedimentation rate (ESR) - with a micro-hematocrit capillary tube (a so-called mini-ESR), as described by Adler and Denton[33]
5. α_1-acid glycoprotein (AGP), otherwise known as orosomucoid - using a gel radial immunodiffusion technique.[55]

The white blood cell count was performed with the Coulter counter and the differential counts by slide evaluation in the routine haematology laboratory.

The protein determinations (IgM, CRP, Hp and AGP) involving immunodiffusion plates or rapid latex technique were performed on materials supplied (and in some cases donated) by Behring Diagnostics. The latex IgM is prepared by the manufacturer to provide positive results at levels of 0.3 g/ℓ or more; the latex Hp at $\geq$ 0.5 g/ℓ (but modified in this study by diluting the antiserum 1:1 to read positive at $\geq$ 0.25 g/ℓ); and the latex CRP at $\geq$ 8 mg/ℓ.

A significant drawback of the immunodiffusion technique is that it takes at least 24 hours to provide an accurate result. The Tarukoski method for measuring haptoglobin is also relatively time-consuming in comparison with the latex technique. However, these tests were evaluated to see if they were more reliable and might require modification in the future.

A scoring system was initially devised (which has been presented in abstract form[59]) with the hope that proven cases would have a high score, probable cases would have intermediate scores and babies without demonstrable infection would have low scores. After initially encouraging results,[59] this was later abandoned, and each test evaluated individually and in combination with other tests. At the end of approximately two and a half years (end of 1977), IgM, α_1-AGP and the Tarukoski evaluation of Hp were discontinued. The remaining tests, whose results could be obtained within an hour, were then applied at the bedside by the housestaff and influenced their decisions about antibiotic use.

The usefulness of diagnostic tests can be estimated from several measures, described most fully by Feinstein[60] and Galen and Gambino,[61] but also considered recently by Card and Emerson.[15] These measures are sensitivity, specificity, positive and negative accuracy (or predictive value) and efficiency. Using the equations obtained from the table

	sepsis	no sepsis
test positive	a	b
test negative	c	d

these may be defined as follows:

Sensitivity - the number of true positive cases (test positive, disease present) divided by the total number of confirmed positive cases; $\frac{a}{(a+c)}$

Specificity - the number of true negative cases (test negative, disease absent) divided by the total number of confirmed negative cases; $\frac{d}{(b+d)}$

Positive predictive accuracy - the number of times the test was correct when the result was positive (true positives/total positives); $\frac{a}{(a+b)}$

Negative predictive accuracy - the number of times the test was correct when the result was negative (true negatives/total negatives); $\frac{d}{(c+d)}$

Efficiency - true positives and true negatives divided by the total evaluated. $\frac{(a+d)}{(a+b+c+d)}$

The standard error for these values may be derived using the formula $SE = \left(\frac{pq}{n}\right)^{\frac{1}{2}}$ where p is the sensitivity or specificity and $q = 1 - p$ and the sample size is n. The 95% confidence limits are calculated as the percent $\pm$ 2 SE. These values are not provided in most tables, for the sake of clarity.

Comparisons of babies evaluated before (1975-77, Group 1) and after (1978-80, Group 2) tests were applied at the bedside were performed using Chi square analysis.

Analysis of babies of differing birth weights (above and below 2500 g) was performed using Student's t test.

RESULTS

> "... the clinician who later uses the test starts with patients whose condition is unknown. The purpose of the test is to predict (or identify) what the patient's condition really is. In receiving the result of the test, an investigator therefore wants to know its predictive accuracy, not its sensitivity or specificity ..."
>
> "... the predictive value will depend entirely on the ratio of confirmed positive and confirmed negative people to whom the test was applied ..."
>
> "... if patients are chosen merely because they do or do not have the target disease, the discrimination of the test will not be adequately evaluated. ... to deal with clinical reality requires a confrontation with clinical complexity ..."
>
> (Feinstein, 1975[60])

There were 524 babies investigated for sepsis during the first 7 days after birth (early group) and 56 babies investigated between 8 and 60 days (late group). In the latter group, 11 were aged 31 to 60 days, and although not strictly "neonatal," they were usually preterm infants evaluated prior to or within a month of their expected date of delivery. In the early group, 298 were males and 226 were females (ratio M:F = 1.3:1). There were 336 babies with birth weights < 2500 grams and the same number less than 37 weeks gestation. There were 41 babies with proven sepsis (23 males, 18 females, ratio M:F = 1.3:1) and 34 babies with "very probable" infection (20 males, 14 females, ratio M:F = 1.4:1). In the late group, the distribution was 37 males, 19 females, and 34 low birth weight, with 12 proven cases of sepsis (9 males, 3 females).

In order to compare the laboratory tests used in diagnosis, it is important to know the incidence of sepsis for each time period and to know the yield of sepsis from various reasons for investigation. It became apparent that "clinical" factors were more predictive than "risk" factors, although a number of babies were investigated for both factors. A breakdown of the yield from individual reasons for investigation is provided in the following Tables*:

Table I - Yield of neonatal sepsis based on investigation for "risk" factors (usually investigated within 48 hours)

0 - 7 DAYS

Risk Factors	Number Investigated (n = 315)	Number with Sepsis (n = 16)	
Prolonged Rupture of Membranes (> 24 h)	140	8	(6%)
Preterm Labour (unexplained)	113	7	(6%)
Maternal Fever/Infection	62	4	(6%)
Foetal Tachycardia	4	1	(25%)
Meconium Stained Amniotic Fluid (unexplained)	20	1	(5%)
Small for Gestational Age	28	0	
Foul-Smelling Amniotic Fluid	23	0	
Prolonged Labour (> 24 h)	1	0	

* - The numbers listed in each table do not add up to the total because some babies were investigated for more than one reason.

Table II - Yield of neonatal sepsis based on investigation for "clinical" factors in the early neonatal period

0 - 7 DAYS

Clinical Factors	Number Investigated (n = 283)	Number with Sepsis (n = 35)
Lethargy	87	23 (26%)
Apnoea (unexplained)	84	11 (13%)
Cyanotic Spells (unexplained)	68	5 (7%)
Temperature Instability	33	4 (12%)
Pustules	6	2 (33%)
Abdominal Distension	13	2 (15%)
Convulsions/Irritability	20	2 (10%)
Jaundice (unexplained)	21	2 (10%)
Poor Feeding	21	1 (5%)
Petechiae/Purpura	12	0
Hepatosplenomegaly	2	0

Table III - Yield of neonatal sepsis based on investigation for "clinical" factors in the late neonatal period.

8 - 60 DAYS

Clinical Factors	Number Investigated (n = 56)	Number with Sepsis (n = 12)
Lethargy	15	5 (33%)
Poor Perfusion	5	3 (60%)
Convulsions/Irritability	8	3 (38%)
Temperature Instability	8	3 (38%)
Poor Feeding	10	3 (30%)
Apnoea	16	2 (13%)
Hepatosplenomegaly	1	1 (100%)
Diarrhoea	2	1 (50%)
Cyanotic Spells	5	1 (20%)
Abdominal Distension	12	1 (8%)
Pustules	5	0
Jaundice	2	0

Some details of the early-group babies with proven sepsis are listed in Table IV. In Table V the details of the early-group babies with very probable infection are provided. In Table VI the late-group babies with proven sepsis are listed.

Table IV - Distribution of positive tests at initial evaluation in babies with documented sepsis in the early neonatal period

Case	Age SWU	B.Wt./GA (g/wks)	Sex	Positive Culture	Causative Organism	Surv. Died	ESR >15	WBC <5000	Latex CRP +ve	I/T Ratio >0.2	Latex Hp +ve
1	3d	2637/36	F	Blood	G.B.S.	S	-	4300	+	0.50	-
2	1d	1984/34	M	Blood	H.influenzae	S	-	-	+	0.34	-
3	2d	2140/33	M	Blood	E.coli	S	-	4100	-	0.22	-
4	3d	3126/37	F	Bl/CSF	E.coli	S	-	4700	+	-	-
5	1h	2910/40	M	Blood	S.pneumoniae	S	-	-	+	0.34	-
6	4h	2240/35	M	Blood	B.subtilis	S	-	-	-	0.61	+
7	6d	1000/28	F	Bl/CSF	B.subtilis	D	-	(72,000)	-	0.44	+
8	3d	3010/36	M	Bl/CSF	E.coli	S	25	2200	+	0.46	-
9	2d	2849/38	M	Bl/CSF	G.B.S.	S	27	-	+	0.65	-
10	1d	1162/33	M	Blood	E.coli	D	(10)	2800	+	0.92	-
11	3h	1340/32	F	Blood	G.B.S.	D	(10)	4600	-	0.31	
12	3d	1520/31	M	Blood	E.coli	D	-	-	-	-	-
13	2d	900/27	F	Blood	E.coli	D	-	-	-	0.23	-
14	4h	3700/40	M	-	Meningitis(? E.coli)Δ	S	19	-	+	0.51	+
15	4d	1180/28	M	Blood	G.B.S.*	S	20	1600	+	-	+
16	1d	2665/35	M	Blood	G.B.S.	D	-	1500	-	1.0**	-
17	7d	2098/35	F	Blood	E.coli	S	33	3200	+	0.47	+
18	2d	1740/31	F	Blood	G.B.S.	D	-	1800	-	1.0**	-
19	3d	2000/36	M	Blood	E.coli	S	25	3700	+	0.24	+
20	6d	2420/44	F	Bl/CSF	E.coli	S	-	-	+	0.44	+
21	14h	1400/32	F	Blood	G.B.S.	D	-	1400	-	1.0**	-
22	2d	2948/40	M	Blood	G.B.S.	S	-	2800	-	0.86	-
23	6d	2900/40	F	Bl/CSF	E.coli	S	32	-	-	0.21	+
24	6h	2360/36	F	Blood	G.B.S.	D	-	3400	-	0.58	-
25	5d	1550/33	F	Bl/CSF	G.B.S.*	S	-	-	+	0.25	-
26	5d	3000/40	M	Bl/CSF	G.B.S.	S	22	-	-	0.30	-
27	7d	5960/40	M	Blood	E.coli*	S	15	-	-	0.32	-
28	1h	1210/31	F	Blood	E.coli	S	-	-	-	0.52	+
29	1h	2960/40	F	Blood	G.B.S.	S	-	-	+	0.48	-
30	3d	1810/34	M	Blood	Staph.aureus	S	-	2800	-	0.37	-
31	2h	2460/36	M	Bl/CSF	G.B.S.	D	-	-	-	0.33	-
32	1d	2790/37	M	Blood	G.D.S.	S	25	-	+	0.39	-
33	12h	2800/37	M	Blood	C.J.	S	(10)	-	-	0.58	+
34	10h	3780/40	F	Blood	E.coli	S	-	-	+	0.26	+
35	2d	2820/36	M	Blood	E.coli	S	-	3200	+	-	-
36	7d	680/28	M	Blood	Staph.epi.	S	-	(36,700)	+	0.25	-
37	5d	2580/37	F	Bl/CSF	Proteus	S	22	-	+	-	+
38	10h	1460/31	M	Blood	G.B.S.	S	-	2500	-	1.0**	-
39	3h	1080/30	M	Urine	E.coli	S	(10)	4600	+	-	-
40	6d	1370/33	F	Bl/CSF	E.coli	S	-	3500	-	0.35	-
41	5d	1100/31	F	Blood	Staph.aureus	S	-	-	+	-	+

* - had negative cultures and tests shortly after birth

** - 1-2 bands, 0 polys

SWU - "sepsis work-up"

Δ - on the basis of CSF cell count, glucose and Gram stain

GBS = group B β-hemolytic streptococci

GDS = group D β-hemolytic streptococci

C.J. = campylobacter jejuni

Staph.epi. = Staphylococcus epidermidis

Table V - Distribution of positive tests at initial evaluation in babies with "very probable" infection in the early neonatal period

Case	Age SWU	B.Wt/GA (g/wks)	Sex	Factors Suggesting Infection	ESR >15	WBC <5000	Latex CRP +ve	I/T Ratio >0.2	Latex Hp +ve
1	2d	3544/40	M	Pneumonia*	-	-	+	-	+
2	8h	2041/35	M	Maternal G.B.S., pneumonia	-	2300	-	0.33	-
3	2h	1956/36	M	Petechiae & purpura, staph. aureus in CSF at 3 days †	-	-	+	0.83	-
4	12h	2840/40	M	Lethargy; trach asp - G.B.S.	-	-	+	0.42	-
5	2d	4111/41	M	Fever, pustule grew staph aureus	-	(24,600)	+	(0.18)	-
6	4d	2280/35	M	Poor feeding; shock; response to antibiotics	25	3200	-	(0.16)	-
7	2h	1300/32	M	PROM; mat. infection & antibiotics; lethargy	17	-	-	0.41	-
8	2d	2060/38	F	Pneumonia; trach asp - G.B.S.	-	3800	+	0.48	-
9	4h	2980/40	F	Apnea; shock; antibiotics prior to blood culture	17	-	+	0.65	-
10	6h	3232/40	F	Fever; maternal G.B.S.	(14)	(40,600)	+	0.59	+
11	4h	4000/38	M	Mat fever; trach asp - E.coli; antibiotics prior to culture	-	(29,000)	+	(0.15)	-
12	3d	2395/39	M	Pneumonia; fever	27	-	+	0.25	+
13	1h	1630/33	M	PROM 48 hrs.; pneumonia	-	-	-	(0.16)	-
14	1h	1970/34	F	PROM; pneumonia	-	-	-	0.43	-
15	2h	2250/36	F	PROM 48 hrs; pneumonia; gram pos. rod at 10 d (bld. culture)†	-	-	+	0.21	-
16	1h	1210/28	M	PROM 2 wks; Apgars $1^1/1^5$	-	4000	+	(0.17)	-
17	1h	1480/30	F	PROM 4 wks; foul-smelling amn. fluid; mat. fever & antibiotic therapy; pneumonia	-	-	+	0.50	-
18	4h	3440/40	F	Apnea; pneumonia with pleural effusion	-	-	+	0.24	-
19	2d	3060/40	M	Apnea; lethargy; pneumonia	-	-	+	-	+
20	2h	2180/34	M	PROM; pneumonia	(10)	-	+	0.22	-
21	0	2830/37	F	Resp. diff.; pneumonia	-	(30,200)	-	-	-
22	0	1400/30	M	PROM; pneumonia; old pleuritis at necropsy; pos. buffy coat smear	-	3200	+	0.39	-
23	12h	930/29	M	Mat. fever; foul-smelling amniotic fluid; apnea	26	-	-	0.75	-
24	1d	4100/38	M	Pneumonia; fever	-	-	-	0.82	+
25	2d	3550/40	F	Lymphangitis	-	(32,200)	+	(0.18)	+
26	1h	2440/35	F	Mat UTI; prem. labor; pneumonia	-	-	+	0.45	-
27	1d	2520/36	F	Resp. diff.; irritable; pneumonia	-	-	+	-	+
28	2h	3860/40	M	PROM; foul-smelling amn. fluid; pneumonia	-	-	+	0.66	-
29	1h	780/28	M	PROM 72 h; pneumonia	30	-	+	0.24	-
30	1h	1240/31	F	PROM 72 h; Mat. cult. - G.B.S. pneumonia	15	-	+	0.43	-
31	3d	1940/35	F	Prem. labor; pneumonia; ET asp. - E. coli	(13)	-	+	0.80	-
32	1d	1930/34	F	Mat. UTI, PROM 48 h; foul-smelling amn. fl.; CSF-bacillus sp. (broth)†	17	-	+	0.40	-
33	1d	1780/33	M	Prem. labor; PROM; pneumonia amniotic fluid - G.B.S.	-	4700	+	0.40	-
34	1d	760/29	M	Mat. fever; apnea; placenta-G.B.S.	28	-	+	0.32	-

SWU = "sepsis work-up"
G.B.S. = Group B β-haemolytic streptococcus
† = Considered to be contaminant organism
* = Pneumonia indicates radiographic infiltrates consistent with this diagnosis

Table VI - Laboratory data at initial evaluation for infants with proved sepsis in the late neonatal period

Patient No./Sex	Birth Weight (g)	Gestation Age (wk)	Age at Evaluation (Days)	Causative Organism	Source	WBC Count <5.0 ($\times 10^9/\ell$)	I/T* Ratio ≥0.2	Latex CRP	Latex Hp	Mini-ESR ≥15 (mm/hr)
1/M	3410	40	20	Staph. aureus	Blood	(32.9)	-	-	+	-
2/M	3487	40	9	Strep. group A	Blood	(27.2)	0.2	-	+	17
3/M	780	33	58	Enterobacter	Blood	4.9	0.32	+	-	42
4/M	2180	35	13	Staph. aureus	Blood	(24.9)	-	+	-	-
5/F†	690	33	13	E. coli	Blood	-	0.4	+	-	-
6/M	2268	40	21	Staph. aureus	Blood, central venous catheter	-	0.21	+	-	25
7/F	750	32	9	Staph. epi.	Blood	3.8	-	+	-	-
8/F	3390	38	33	E. coli	Blood, urine	-	-	+	-	30
9/M†	4280	40	18	Strep. group B	Blood, CSF	4.0	0.58	+	-	-
10/M	3720	40	47	Haemophilus influenzae	Blood, CSF	3.9	0.77	+	-	-
11/M	780	28	12	Strep. group D	Urine	-	-	-	+	25
12/M	2700	37	13	Pseudomonas aeruginosa	Blood	-	0.49	+	-	20

* = I/T ratio indicates immature/total neutrophils
† = Died
Staph. epi. = Staphylococcus epidermidis
CRP = C-reactive protein
Hp = Haptoglobin

Leucocyte Count

Because many different values of total leucocyte count, neutrophil count, band count and ratios (or fractions) of immature and segmented neutrophils have been described, the leucocyte count was analyzed in several different ways for the early group. These are provided in Table VII. Numbers were too small in the late group to provide useful analysis.

By careful perusal of Table VII, it can be seen that some values are more sensitive than others, but lose something either in specificity or predictive value. The most useful levels seem to be total leucocyte count of less than $5.0 \times 10^9/\ell$ (sensitivity 19/41, 46%; positive predictive value 19/48, 40%) and an I/T ratio of ≥ 0.2 (sensitivity 34/41, 83%; positive predictive value 34/140, 24%). However, it is evident that a total leucocyte count of $< 10.0 \times 10^9/\ell$ and an I/T ratio of ≥ 0.15 seem to be the most sensitive indicators (30/41, 73% and 36/41, 88% respectively), without losing too much in specificity. Elevated leucocyte counts only seem to be of value at the end of the first week. Figure 1 shows the values for total leucocyte counts according to infection status by day of age of evaluation in low birth weight infants. In Figure 2 infants with proven sepsis are plotted by wbc and I/T ratio. Figure 3 shows the I/T ratio plotted against birth weight in infants with sepsis.

Table VII - The predictive value for sepsis of several leucocyte levels suggested as indicative of neonatal infection, at different ages during the first week after birth

	Day after Birth					
	0	1	2	3-4	5-7	Total
Total Evaluated	296	110	40	50	28	524
No. with proven sepsis	^{8} 3%	^{9} 8%	^{7} 18%	^{7} 14%	^{10} 36%	^{41} 8%
Leucocytes						
$\geq 20.0 \times 10^9/\ell$	$^{0}/_{50}$	$^{0}/_{27}$	$^{0}/_{5}$	$^{0}/_{1}$	$^{2}/_{6}$ 33%	$^{2}/_{89}$* 2%
$\geq 25.0 \times 10^9/\ell$	$^{0}/_{24}$	$^{0}/_{9}$	$^{0}/_{2}$	$^{0}/_{0}$	$^{2}/_{2}$ 100%	$^{2}/_{37}$ 5%
$\geq 30.0 \times 10^9/\ell$	$^{0}/_{11}$	$^{0}/_{3}$	$^{0}/_{1}$	$^{0}/_{0}$	$^{2}/_{2}$ 100%	$^{2}/_{17}$ 12%
$< 10.0 \times 10^9/\ell$	$^{6}/_{93}$ 6%	$^{7}/_{40}$ 18%	$^{6}/_{22}$ 27%	$^{7}/_{34}$ 21%	$^{4}/_{11}$ 36%	$^{30}/_{200}$ 15%
$< 5.0 \times 10^9/\ell$	$^{2}/_{16}$ 13%	$^{5}/_{10}$ 50%	$^{5}/_{7}$ 71%	$^{5}/_{13}$ 38%	$^{2}/_{2}$ 100%	$^{19}/_{48}$ 40%
$< 4.0 \times 10^9/\ell$	$^{0}/_{5}$	$^{5}/_{7}$ 71%	$^{4}/_{6}$ 67%	$^{3}/_{8}$ 38%	$^{2}/_{2}$ 100%	$^{14}/_{28}$ 50%
Neutrophils						
$> 10.0 \times 10^9/\ell$	$^{0}/_{79}$	$^{0}/_{44}$	$^{0}/_{11}$	$^{0}/_{5}$	$^{3}/_{9}$ 33%	$^{3}/_{148}$ 2%
$> 15.0 \times 10^9/\ell$	$^{0}/_{24}$	$^{0}/_{14}$	$^{0}/_{5}$	-	$^{2}/_{3}$ 67%	$^{2}/_{45}$ 4%
$< 1.5 \times 10^9/\ell$	$^{3}/_{21}$ 14%	$^{3}/_{8}$ 38%	$^{3}/_{3}$ 100%	$^{2}/_{8}$ 25%	$^{2}/_{3}$ 67%	$^{13}/_{43}$ 30%
$< 1.0 \times 10^9/\ell$	$^{3}/_{12}$ 35%	$^{3}/_{6}$ 50%	$^{2}/_{2}$ 100%	$^{2}/_{6}$ 33%	$^{1}/_{1}$ 100%	$^{11}/_{27}$ 41%
Immature Neutrophils						
$> 1.5 \times 10^9/\ell$	$^{1}/_{56}$ 2%	$^{4}/_{39}$ 10%	$^{1}/_{7}$ 14%	$^{0}/_{6}$	$^{4}/_{5}$ 80%	$^{10}/_{113}$ 9%
$> 1.0 \times 10^9/\ell$	$^{4}/_{82}$ 5%	$^{4}/_{52}$ 8%	$^{1}/_{10}$ 10%	$^{1}/_{8}$ 13%	$^{5}/_{6}$ 83%	$^{15}/_{158}$ 9%
Immature/Total Neutrophil Ratio						
≥ 0.15	$^{7}/_{82}$ 9%	$^{9}/_{56}$ 16%	$^{6}/_{18}$ 33%	$^{6}/_{16}$ 38%	$^{8}/_{9}$ 89%	$^{36}/_{181}$ 20%
≥ 0.20	$^{7}/_{63}$ 11%	$^{9}/_{45}$ 20%	$^{6}/_{12}$ 50%	$^{4}/_{11}$ 36%	$^{8}/_{9}$ 89%	$^{34}/_{140}$ 24%
≥ 0.30	$^{7}/_{41}$ 17%	$^{6}/_{28}$ 21%	$^{4}/_{7}$ 57%	$^{2}/_{6}$ 33%	$^{6}/_{6}$ 100%	$^{25}/_{88}$ 28%

* - By providing the individual numbers, the values for sensitivity and specificity can be derived. For example, from $^{2}/_{89}$ we know that sensitivity is $^{2}/_{41}$ (5%) and specificity is $^{396}/_{483}$ (82%).

Figure 1 - The total leucocyte count at the time of initial evaluation for sepsis is shown in infants with birth weight less than 2500 grams, according to infection status at different times of evaluation.

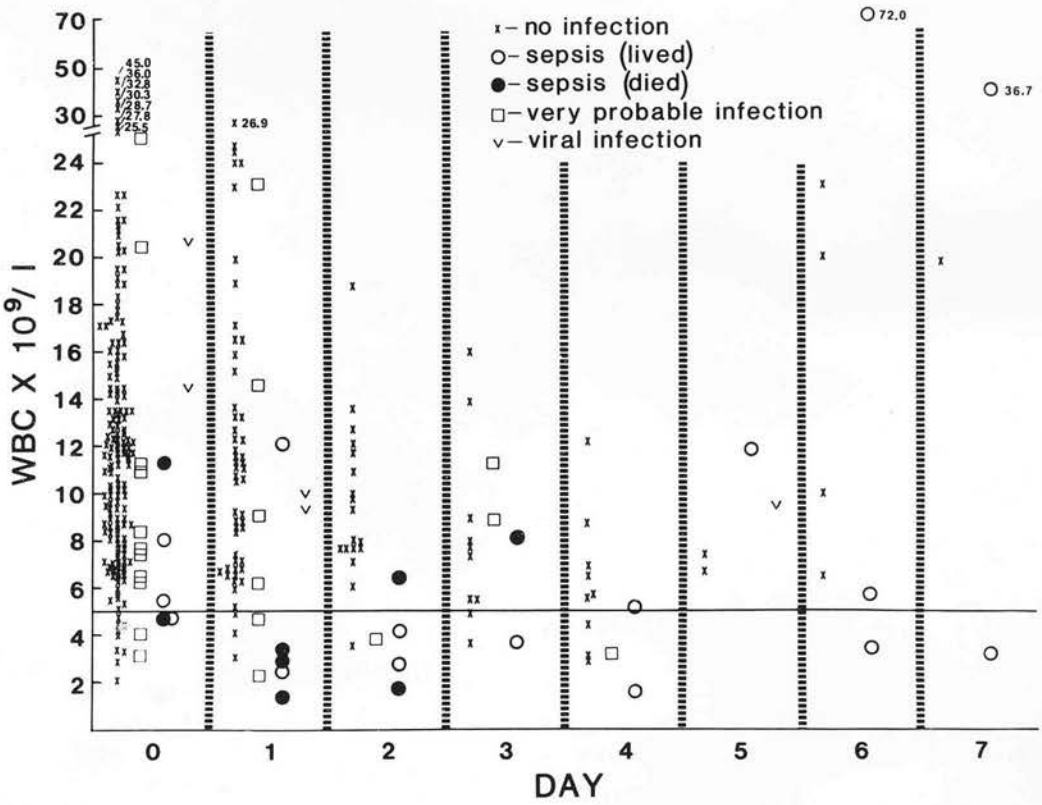


Figure 2 - The immature to total neutrophil ratio is plotted against the total leucocyte count in infants with proven sepsis during the first week after delivery.

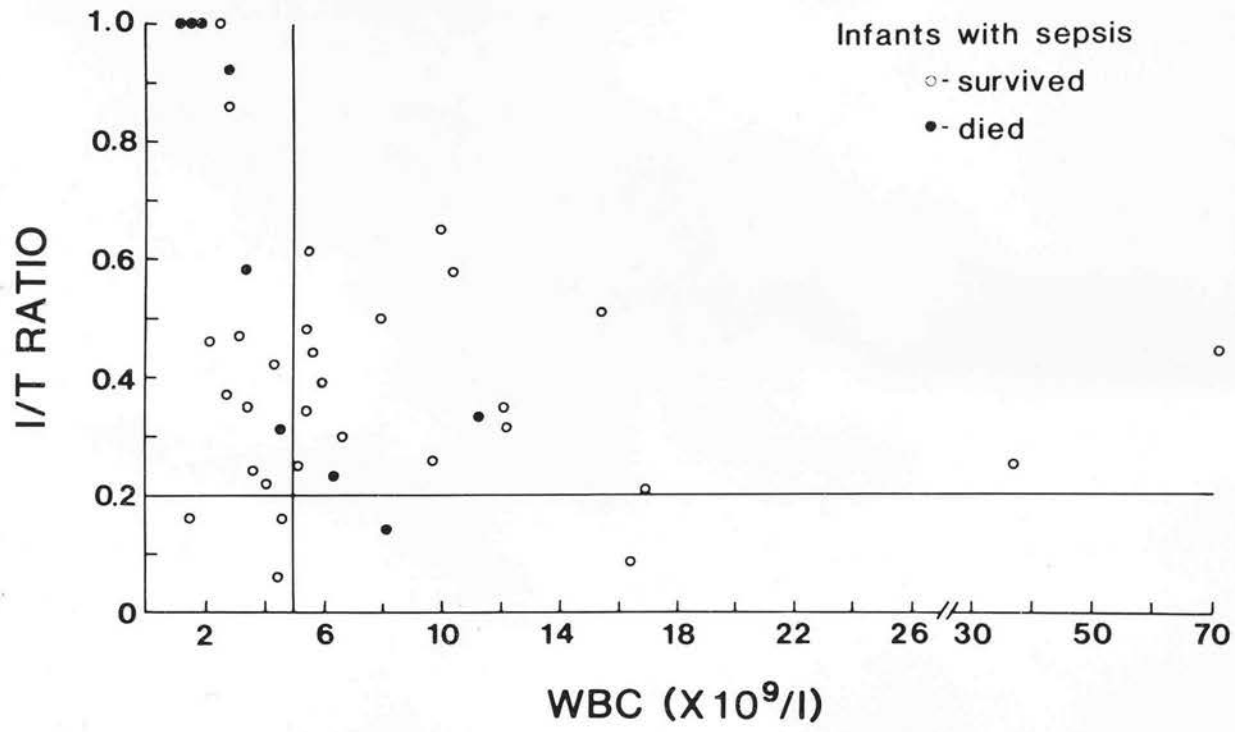
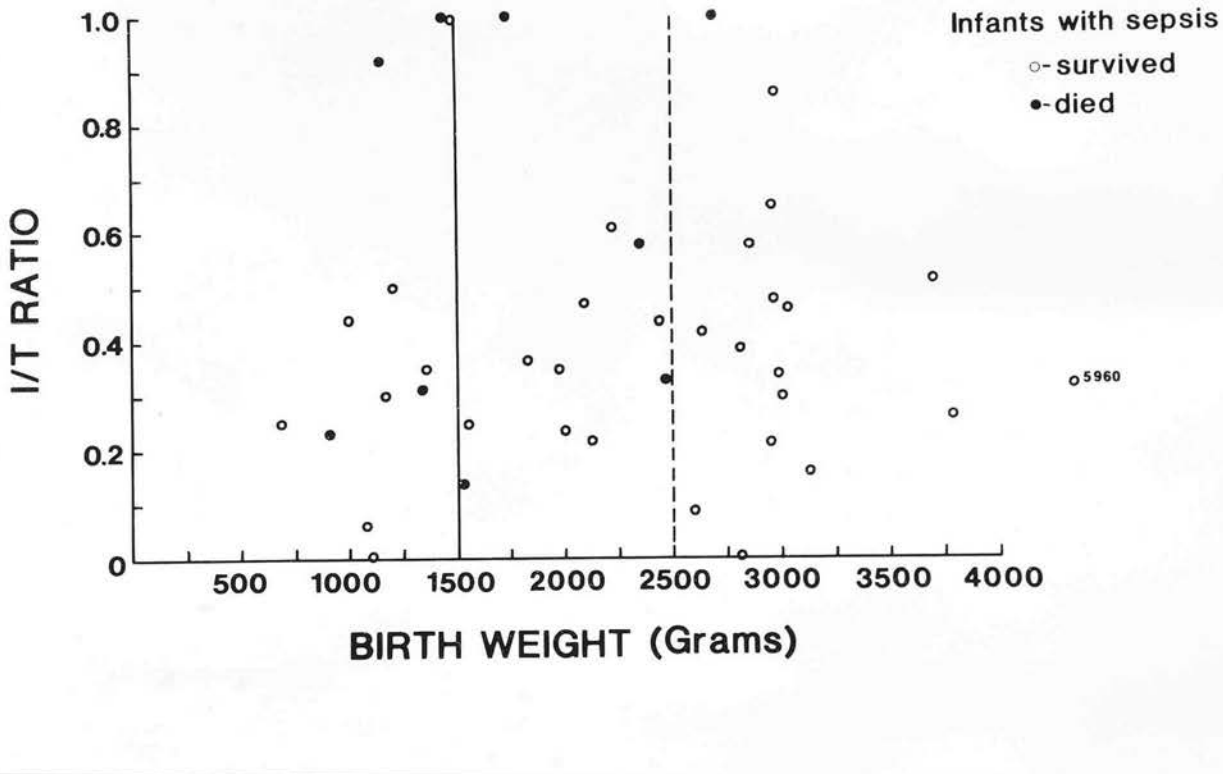


Figure 3 - The immature to total neutrophil ratio is plotted against birth weight in infants with proven sepsis during the first week after delivery.



When non-infected infants with birth weights above and below 2500 grams were compared, the low birth weight infants had significantly lower levels of total leucocytes, segmented neutrophils and immature neutrophils, but the I/T ratio was not affected (Table VIII).

Table VIII - Mean (± SEM) values for various leucocyte counts in non-infected infants with birth weights above and below 2500 grams.

Age	Test ($\times 10^9/\ell$)	Birth Weight < 2500 g	Birth Weight > 2500 g	p value (t test)
0 - 7 DAYS		n = 218	n = 143	
	Total WBC	11.6 ± 0.43	17.3 ± 0.59	< 0.001
	Segmented Neutrophils	5.2 ± 0.27	9.9 ± 0.43	< 0.001
	Band Neutrophils	0.76 ± 0.09	1.5 ± 0.15	< 0.001
	Immature/Total Neutrophils	0.12 ± 0.01	0.13 ± 0.01	n.s.
0 - 6 HOURS		n = 131	n = 72	
	Total WBC	12.0 ± 0.58	18.2 ± 0.87	< 0.001
	Segmented Neutrophils	5.2 ± 0.34	10.3 ± 0.66	< 0.001
	Band Neutrophils	0.75 ± 0.13	1.6 ± 0.23	< 0.01
	Immature/Total Neutrophils	0.10 ± 0.01	0.14 ± 0.02	n.s.
DAY 1		n = 47	n = 43	
	Total WBC	12.1 ± 0.91	18.1 ± 0.98	< 0.001
	Segmented Neutrophils	5.8 ± 0.69	10.3 ± 0.74	< 0.001
	Band Neutrophils	0.89 ± 0.15	1.7 ± 0.27	< 0.02
	Immature/Total Neutrophils	0.15 ± 0.03	0.15 ± 0.02	n.s.
DAY 2		n = 16	n = 10	
	Total WBC	9.8 ± 0.89	15.5 ± 2.05	< 0.02
	Segmented Neutrophils	5.1 ± 0.68	9.0 ± 1.39	< 0.02
	Band Neutrophils	0.73 ± 0.35	1.3 ± 0.56	n.s.
	Immature/Total Neutrophils	0.11 ± 0.03	0.12 ± 0.04	n.s.

Erythrocyte Sedimentation Rate

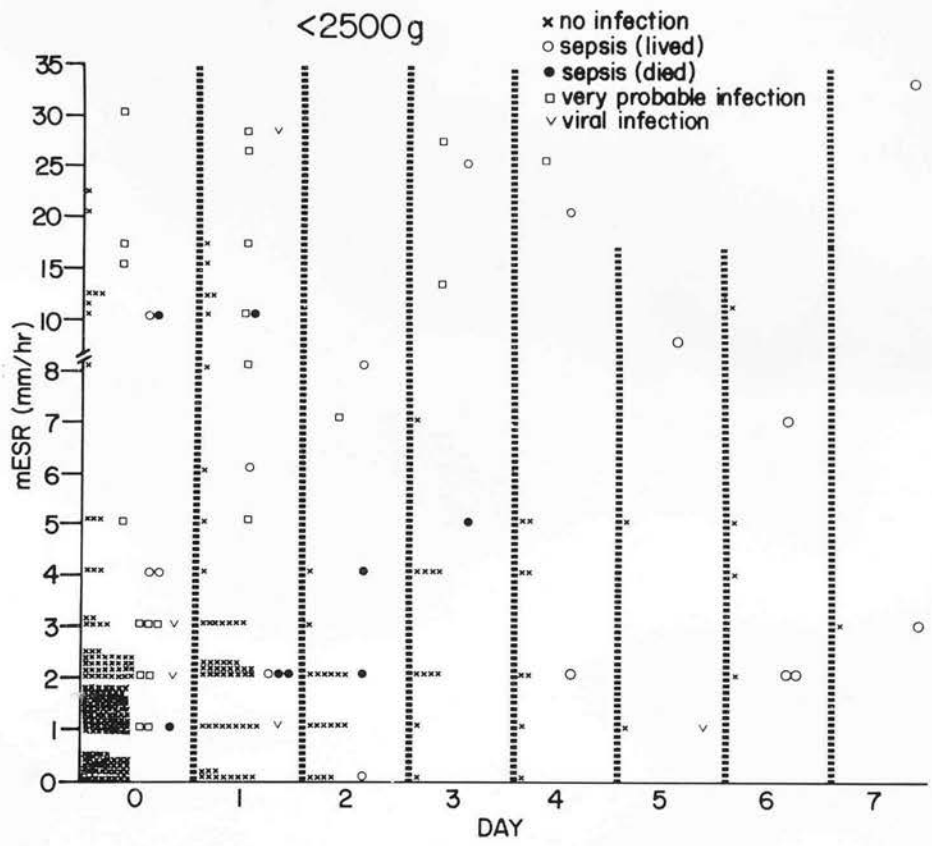
The modification used in this study was previously dubbed the mini-ESR.[32] Normal values on the first day are reported to be 0-2 mm/h and may rise as high as 10 mm/h by the end of the first week.[32,62]

Results of the mini-ESR for different groups of babies (sepsis, "very probable" and "not infected") are provided in Table IX according to the day of evaluation. Figure 4 shows the distribution of values in low birth weight infants according to infection status by day of age at evaluation. In view of the low normal values on the first two days after birth, it may be more useful to use levels $\geq$ 10 mm/h as abnormal. This certainly improves sensitivity, but predictive accuracy suffers in consequence.

Table IX - Erythrocyte sedimentation rate by day of evaluation and infection status

	ESR (Mean ± SEM)					
Age	n	"Not Infected"	n	"Very Probable"	n	"Proven"
0 - 6 hours	272	1.9 ± 0.2	16	6.4 ± 2.1	8	7.4 ± 2.1
1 day	90	2.5 ± 0.3	11	10.9 ± 2.8	9	6.8 ± 2.5
2 days	29	1.7 ± 0.2	4	6.5 ± 1.0	7	6.1 ± 3.6
3 - 4 days	40	3.7 ± 0.5	3	22.0 ± 7.0	7	12.6 ± 4.8
5 - 7 days	18	6.4 ± 2.0	0		10	14.7 ± 4.7
Total (0 - 7 d)	449	2.3 ± 0.2	34	9.2 ± 1.6	41	9.7 ± 1.5

Figure 4 - The distribution of mini-ESR values in low birth weight infants is shown according to infection status by day of age at evaluation.



C-Reactive Protein

The latex CRP test provides semiquantitative results, with a positive indicating levels of CRP of greater than 8 mg/ℓ. Therefore, it is not possible to plot the levels in the same way as leucocytes and ESR. Table X shows the frequency of positive tests by day of evaluation according to infection status.

Table X - Frequency of positive latex C-reactive protein tests by day of evaluation and infection status.

Age	Not Infected	"Very Probable"	Proven	Total
0 - 6 hours	17	12	4	33
1 day	17	8	5	30
2 days	2	4	2	8
3 - 4 days	6	2	6	14
5 - 7 days	3	0	5	8
Total 1st week	45	26	22	93
8 - 30 days	4	3	6	13
31 - 60 days	3	3	3	9
TOTAL	52	32	31	115

Haptoglobin

Early in the study it became apparent that the time consuming technique of determining haptoglobin by the Tarukoski method gave little more information than that provided by the modified latex haptoglobin test, which gives a positive at levels greater than 0.25 g/ℓ. Table XI shows the frequency of positive tests by day of evaluation according to infectious status. In older infants (8-60 days) the latex haptoglobin does not require modification and provides a positive at 0.5 g/ℓ.

Table XI - Frequency of positive latex haptoglobin tests by day of evaluation and infection status

Age	Not Infected	"Very Probable"	Proven	Total
0 - 6 hours	7	0	3	10
1 day	4	4	2	10
2 days	1	2	0	3
3 - 4 days	3	1	3	7
5 - 7 days	1	0	6	7
Total 1st week	16	7	14	37
8 - 30 days	4	2	3	9
31 - 60 days	0	1	0	1
TOTAL	20	10	17	47

α_1-Acid Glycoprotein (Orosomucoid)

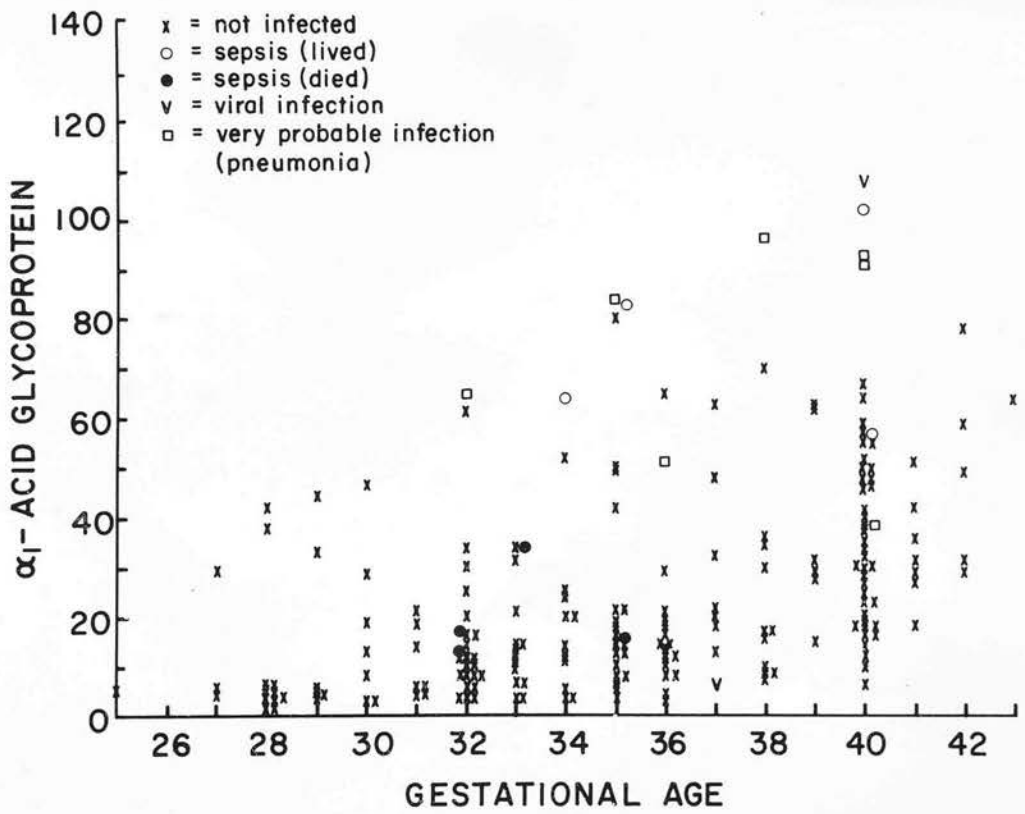
Levels of α_1-acid glycoprotein were measured routinely in the first half of the study, but because of the delay in availability of the results, it was decided that other tests were more useful. However, this protein has potential value, provided a rapid technique of measurement is available. Such a technique is now available.[51] Because of this, the results on the first 278 babies are presented in Table XII according to age and infection status. The infected groups have significantly higher levels ($p < 0.0001$) than the "non-infected," when all ages are combined. The survivors of sepsis ($n = 14$) had a mean α_1-AGP level of 90 mg/dl, whereas those who died of sepsis ($n = 7$) had a mean α_1-AGP level of 19 mg/dl. One other baby died subsequently and had an initial level of 48 mg/dl. The number of babies investigated in the "not infected" group decreases markedly with advancing post-natal age, but there seems to be a gradual increase of α_1-AGP with increasing post-natal age. Although the "very probable" group had significantly higher levels ($p < 0.001$) for each time period, the proven sepsis group was not different on Days 1 and 2. This may be explained in part by the fact that those babies with sepsis who subsequently died had lower levels than those who survived.[13] Three babies died on Day 1 with α_1-AGP levels of 34, 15 and 13 mg/dl, and two died on Day 2 with levels of 30 and 14 mg/dl.

Table XII - Levels of α_1-acid glycoprotein at various ages during the first week after delivery according to infection status.

	α_1-acid glycoprotein (mean ± SEM) in mg/dl					
Age	n	"Not Infected"	n	"Very Probable"	n	Proven Sepsis
0 - 6 hours	125	23 ± 1.6	4	77 ± 11.0	4	65 ± 18
1 day	72	25 ± 2.3	3	71 ± 16.0	4	32 ± 12
2 days	20	29 ± 4.2	3	63 ± 2.4	5	35 ± 10
3 - 4 days	20	41 ± 4.0	2	134 ± 27.0	6	83 ± 14
5 - 7 days	7	50 ± 11.0	0		3	129 ± 44

In Figure 5 the α_1-AGP values obtained in the first 24 hours after delivery are plotted against gestational age, according to infection status. Although some infants subsequently considered to be "not infected" had high levels, the majority of high levels were in infants with sepsis (who survived) or in those with very probable infection. There was a statistically significant correlation between gestational age and levels of α_1-acid glycoprotein in the "not infected" group ($r = 0.452$, $p < 0.001$).

Figure 5 - The levels of α_1-acid glycoprotein (in mg/dl) are plotted against gestational age for all babies evaluated within 24 hours of delivery. Levels above 50 mg/dl are considered abnormal at this age.



Tests of Limited Value

In the first half of the study, platelet counts and IgM levels were routinely evaluated and gastric aspirate smears were examined when indicated (e.g., following prolonged rupture of membranes). Because of the following results, these tests were "abandoned" and are not considered in further analyses.

Platelet Count - Platelet counts of less than $150 \times 10^9/\ell$ were considered abnormal. There were very few abnormal counts when the babies were first evaluated. Indeed, only 15 babies had abnormal counts and only 2 were found to have sepsis. A number of babies did develop low platelet counts later in their course.

Immunoglobulin M - A level exceeding 0.3 g/ℓ was considered abnormal, since this was the limit for the latex reagent (designed to detect abnormal levels). Of 45 infants with an IgM level > 0.3 g/ℓ (using radial immunodiffusion), only 3 infants proved to have sepsis.

Gastric Aspirate Smear - 101 babies had gastric contents aspirated and sent for smear. The presence of 5 or more polymorphonuclear leucocytes per high-power field was considered abnormal. There were 20 babies with positive smears, of whom 4 were infected. Of 81 babies with negative smears, 4 were infected. These findings were not significant (χ^2 with Yates correction = 3.14, $p > 0.05$).

Combination of Tests

In evaluating individual tests, it became apparent that although individual tests might have great sensitivity or specificity for sepsis or "infection," it was rare to find both elevated. Even more important from the clinician's point of view was the fact that the tests with the greatest sensitivity (I/T ratio and latex CRP) had the poorest positive predictive accuracy. Because of this, various combinations were evaluated. The most satisfactory combination seemed to be two or more of five tests. In addition, using either I/T ratio ≥ 0.2 and/or wbc $< 5.0 \times 10^9/\ell$ had high sensitivity.

Details of sensitivity, specificity, predictive value and efficiency are provided in Table XIII for the five tests individually and the two combinations just mentioned. Details are provided for both sepsis detection and "infection" detection, in babies evaluated during the first week after birth. Further details for positive predictive value by age at evaluation in infants of low birth weight are provided in Table XIV.

Table XIII - Sensitivity, specificity, predictive value and efficiency of several tests for sepsis and "infection"*

	Sensitivity $(\frac{a}{a+c})$	Specificity $(\frac{d}{b+d})$	Positive Predictive Value $(\frac{a}{a+b})$	Negative Predictive Value $(\frac{d}{c+d})$	Efficiency $(\frac{a+d}{a+b+c+d})$
Sepsis					
I/T ≥ 0.2	$^{34}/_{41}$ 83%	$^{376}/_{483}$ 78%	$^{34}/_{141}$ 24%	$^{376}/_{383}$ 98%	$^{410}/_{524}$ 78%
WBC < $5.0 \times 10^9/\ell$	$^{19}/_{41}$ 46%	$^{453}/_{483}$ 94%	$^{19}/_{49}$ 39%	$^{453}/_{475}$ 95%	$^{472}/_{524}$ 90%
CRP Positive	$^{22}/_{41}$ 54%	$^{412}/_{483}$ 85%	$^{22}/_{93}$ 24%	$^{412}/_{431}$ 96%	$^{434}/_{524}$ 83%
Hp Positive	$^{14}/_{41}$ 34%	$^{460}/_{483}$ 95%	$^{14}/_{37}$ 38%	$^{460}/_{487}$ 94%	$^{474}/_{524}$ 90%
ESR ≥ 15 mm/h	$^{11}/_{41}$ 27%	$^{464}/_{483}$ 96%	$^{11}/_{29}$ 38%	$^{464}/_{494}$ 94%	$^{475}/_{524}$ 91%
Any two or more	$^{38}/_{41}$ 93%	$^{422}/_{483}$ 87%	$^{38}/_{99}$ 38%	$^{422}/_{426}$ 99%	$^{460}/_{524}$ 88%
I/T ≥ 0.2 ± WBC < 5.0	$^{38}/_{41}$ 93%	$^{359}/_{483}$ 74%	$^{38}/_{162}$ 23%	$^{359}/_{362}$ 99%	$^{397}/_{524}$ 76%
(Incidence of sepsis is $^{41}/_{524}$ = 7.8%)					
"Infection"*					
I/T ≥ 0.2	$^{59}/_{75}$ 79%	$^{367}/_{449}$ 82%	$^{59}/_{141}$ 42%	$^{367}/_{387}$ 95%	$^{426}/_{524}$ 81%
WBC < $5.0 \times 10^9/\ell$	$^{25}/_{75}$ 33%	$^{425}/_{449}$ 95%	$^{25}/_{49}$ 51%	$^{425}/_{475}$ 89%	$^{450}/_{524}$ 86%
CRP Positive	$^{48}/_{75}$ 64%	$^{404}/_{449}$ 90%	$^{48}/_{93}$ 52%	$^{404}/_{431}$ 94%	$^{452}/_{524}$ 86%
Hp Positive	$^{21}/_{75}$ 28%	$^{433}/_{449}$ 96%	$^{21}/_{37}$ 57%	$^{433}/_{487}$ 89%	$^{454}/_{524}$ 87%
ESR ≥ 15 mm/h	$^{20}/_{75}$ 27%	$^{439}/_{449}$ 98%	$^{20}/_{29}$ 69%	$^{439}/_{494}$ 89%	$^{459}/_{524}$ 88%
Any two or more	$^{67}/_{75}$ 89%	$^{417}/_{449}$ 93%	$^{67}/_{99}$ 68%	$^{417}/_{426}$ 98%	$^{484}/_{524}$ 92%
I/T ≥ 0.2 ± WBC < 5.0	$^{64}/_{75}$ 85%	$^{351}/_{449}$ 78%	$^{64}/_{162}$ 40%	$^{351}/_{362}$ 97%	$^{415}/_{524}$ 79%
(Incidence of "infection" is $^{75}/_{524}$ = 14.3%)					

* - "Infection" = proven sepsis plus "very probable" infection

Table XIV - Predictive value of several tests for sepsis at different ages after birth in low birth weight infants

< 2500 g	0 d	1 d	2 d	3 - 4 d	5 - 7 d	Total
Total Evaluated	208	65	23	26	14	336
Sepsis Proved	5	5	4	4	6	24
"Very Probable"	11	6	1	3	0	21
"Not Infected"	192	54	18	19	8	291
WBC < $5.0 \times 10^9/\ell$ Sepsis/Total	$^{2}/_{14}$ 14%	$^{4}/_{9}$ 44%	$^{3}/_{5}$ 60%	$^{2}/_{8}$ 25%	$^{2}/_{2}$ 100%	$^{13}/_{38}$ 34%
I/T Ratio ≥ 0.2 Sepsis/Total	$^{4}/_{39}$ 10%	$^{5}/_{29}$ 17%	$^{4}/_{7}$ 57%	$^{2}/_{9}$ 22%	$^{5}/_{6}$ 83%	$^{20}/_{90}$ 22%
Latex CRP Positive Sepsis/Total	$^{1}/_{20}$ 5%	$^{2}/_{14}$ 14%	$^{0}/_{3}$ 0%	$^{3}/_{6}$ 50%	$^{4}/_{5}$ 80%	$^{10}/_{48}$ 21%
Latex Hp Positive Sepsis/Total	$^{2}/_{5}$ 40%	$^{0}/_{1}$ 0%	$^{0}/_{0}$ -	$^{2}/_{3}$ 67%	$^{4}/_{4}$ 100%	$^{8}/_{13}$ 62%
ESR ≥ 15 mm/h Sepsis/Total	$^{0}/_{5}$ 0%	$^{0}/_{6}$ 0%	$^{0}/_{0}$ -	$^{2}/_{4}$ 50%	$^{1}/_{1}$ 100%	$^{3}/_{16}$ 19%
2 or More Positive Sepsis/Total	$^{4}/_{20}$ 20%	$^{5}/_{19}$ 26%	$^{3}/_{4}$ 75%	$^{3}/_{8}$ 38%	$^{6}/_{7}$ 86%	$^{21}/_{58}$ 36%
2 or More Positive "Infection"/ Total	$^{13}/_{20}$ 65%	$^{11}/_{19}$ 58%	$^{4}/_{4}$ 100%	$^{6}/_{8}$ 75%	$^{6}/_{7}$ 86%	$^{40}/_{58}$ 69%

In Table XV details of sensitivity for sepsis are provided for the five tests and the combination of two or more of these five tests (designated as a "sepsis screen"), according to age at evaluation and birth weight. It is noteworthy that all infants with birth weight $\geq$ 2500 grams who had sepsis were detected with the sepsis screen, but three infants with birth weight < 2500 grams were "missed." These three babies require some brief explanation. All three died, and in two of them the positive blood cultures were obtained within six hours of death. One had necrotising enterocolitis, while the second had severe respiratory distress syndrome and no evidence of infection at necropsy. The third baby had overwhelming group B streptococcal sepsis with a normal total leucocyte count, but profound neutropenia. All three received antibiotics immediately after their blood cultures were drawn.

One might suspect that increased predictive value would be obtained with an increasing number of tests positive. This is suggested in Table XVI, but the numbers with multiple tests positive are small.

The usefulness of the combination of tests described is supported by the data in infants between 8-60 days. These data are presented in Tables XVII and XVIII.

Table XV - Sensitivity of tests for sepsis according to age at evaluation and birth weight category

		Sepsis Proven	Number of Infants with Sepsis with					
			WBC < 5.0	I/T Ratio	CRP +ve	Hp +ve	ESR > 15	2 or More
0-6h	<2500g	5	2	4	1	2	0	4
	≥2500g	3	0	3	3	1	1	3
1d	<2500g	5	4	5	2	0	0	5
	≥2500g	4	1	4	3	2	1	4
2d	<2500g	4	3	4	0	0	0	3
	≥2500g	3	2	2	2	0	1	3
3-4d	<2500g	4	2	2	3	2	2	3
	≥2500g	3	3	2	3	1	1	3
5-7d	<2500g	6	2	5	4	4	1	6
	≥2500g	4	0	3	1	2	4	4
0-7d	<2500g	24	13	20	10	8	3	21
	≥2500g	17	6	14	12	6	8	17

Table XVI - Sepsis screen scores for infants evaluated in the first week

	Score of Sepsis Screen,* No. (%)				
	0 - 1	2	3	4	5
Sepsis	3 (1)	24 (34)	8 (39)	4 (67)	2 (100)
Viral Infection	3 (1)	3 (4)	1 (5)	0	0
Very Probable Infection	5 (1)	19 (28)	9 (43)	1 (17)	0
Not Infected	415 (97)	23 (33)	3 (14)	1 (17)	0
TOTAL	426	69	21	6	2

* - The score is derived from the presence of five diagnostic findings (WBC count < 5.0 x 10^9/ℓ; immature/total neutrophils ≥ 0.2; ESR ≥ 15 mm/h; latex CRP positive; and latex Hp positive).

Table XVII - The value of individual tests compared with a combination of tests in 12 proved cases of neonatal sepsis from 56 infants evaluated between 8 and 60 days of age

Test*	Total +ve Tests	+ve Tests with Proved Sepsis	+ve Predictive Accuracy, % (± SE)	Sensitivity,† % (± SE)	Specificity Δ % (± SE)
WBC < 5.0×10^9/ℓ	7	4	57 (± 7)	33 (± 6)	90 (± 4)
I/T Ratio ≥ 0.2	17	7	41 (± 7)	58 (± 7)	79 (± 5)
Latex CRP +ve	22	9	41 (± 7)	75 (± 6)	71 (± 6)
Latex Hp +ve	10	3	30 (± 6)	25 (± 6)	86 (± 5)
Mini-ESR ≥ 15 mm/h	14	6	43 (± 7)	50 (± 7)	83 (± 5)
Sepsis screen +ve ∇ (any 2 or more)	23	10	43 (± 7)	83 (± 5)	74 (± 6)
WBC ≥ 20.0×10^9/ℓ	6	3	50 (± 7)	25 (± 6)	88 (± 4)
Modified Sepsis Screen +ve	26	12	46 (± 7)	100	69 (± 6)

* - I/T Ratio - Indicates immature/total neutrophils,
CRP - C-reactive protein,
Hp - Haptoglobin.

† - Sensitivity - If disease present, test results positive.

Δ - Specificity - If disease absent, test results negative (Two cases of viral infection are excluded).

∇ - +ve - Positive indicates 2 or more of the 5 diagnostic tests having positive results.

Table XVIII - Sepsis screen scores for infants aged between 8 and 60 days

	Score of Sepsis Screen,* No. (%)				
	0-1	2	3	4	5
Sepsis	2 (6)	4 (29)	5 (71)	1 (50)	0
Viral Infection	0	2 (14)	0	0	0
Very Probable Infection †	0	5 (36)	2 (29)	0	0
Superficial Infection	5 (15)	2 (14)	0	0	0
Not Infected	26 (79)	1 (7)	0	1 (50)	0
TOTAL	33	14	7	2	0

* - The score is derived from the presence of five diagnostic findings (see Table XVI).

† - Includes two cases of necrotising enterocolitis.

Although the combination of tests seems to be valuable throughout the neonatal period, the results of the tests are presented by age at evaluation in Table XIX.* The predictive value increases with increasing post-natal age*, but it should be appreciated that this probably reflects an increasing incidence of infection in those evaluated later. Perhaps a better way to interpret these results is to consider the incidence at each age and to consider the improvement in predictive accuracy. For instance, for 0-6 hours, the incidence of sepsis is 3%, so that with no previous knowledge of the tests, one would correctly predict sepsis 3% of the time; but with the "sepsis screen" positive, sepsis is correctly predicted in 23% (an improvement of more than seven times). On the other hand at 5-7 days, 36% of the babies evaluated had sepsis (making "a priori" predictiveness 36%), but the sepsis screen had a positive predictive value of 83% (an improvement of approximately two and a half times).

Table XIX - Positive predictive accuracy of positive "sepsis screen" for sepsis and "infection" at different ages

	Sepsis		Infection	
0 - 1 day	16/62	26%	39/62	63%
2 - 7 days	22/37	59%	28/37	76%
8 - 30 days	7/15	47%	12/15	80%
31 - 60 days	3/8	38%	6/8	75%
Total	48/122	39%	85/122	70%
Total Sepsis/ Total Evaluated	53/580	9%	82/580	14%

* - see also Table XIV

Since not all tests may be available, it may be useful to know the predictiveness of various combinations. These are provided in Tables XX and XXI.

Table XX - The predictive value of different pairs of tests for sepsis in the early neonatal period

0 - 7 DAYS

Combination of Tests	Total Positive	Positive Score Proven Infection	Positive Predictive Accuracy	False* Positives
WBC and I/T Ratio	22	15	68%	3
CRP and I/T Ratio	50	15	30%	10
Hp and I/T Ratio	18	10	56%	2
ESR and I/T Ratio	21	9	43%	2
WBC and CRP	14	9	64%	1
ESR and CRP	19	8	42%	2
Hp and CRP	22	8	36%	3
Hp and ESR	9	6	67%	0
WBC and ESR	5	4	80%	1
WBC and Hp	5	3	60%	2
Any 2 or More	99	38/41 (93%)	38/99 (38%)	27/442 (6%)

* - Includes positive combination in "not infected" group, excludes "very probable" group.

Table XXI - The predictive value of different pairs of tests for sepsis in the late neonatal period

8 - 60 DAYS

Combination of Tests	Total Positive	Positive Score Proven Infection	Positive Predictive Accuracy	False† Positives
WBC and I/T Ratio	10	6	60%	2
WBC and CRP	6 (+2)*	4 (+1)	67% (63%)	1
ESR and CRP	8	4	50%	1
ESR and I/T Ratio	7	4	57%	1
WBC and I/T Ratio	6 (+2)	3 (+1)	50% (50%)	1
Hp and ESR	3	2	67%	0
WBC and ESR	3 (+1)	1 (+1)	33% (50%)	1
Hp and I/T Ratio	2	1	50%	0
WBC and Hp	0 (+5)	0 (+2)	- (40%)	0 (+1)
Hp and CRP	2	0	0%	0
Any 2 or More	23 (+3)	10 (+2)	43% (46%)	2 (+1)
% of Total (n=56)	41% (46%)	83% (100%)		4% (5%)

† - Excludes "very probable" group

* - Figures in parentheses indicate the addition of WBC $\geq 20.0 \times 10^9/\ell$

In a few cases, sequential measurements were carried out. There were seven babies where repeat studies were carried out approximately 24 hours after initial evaluation. The results are presented in Table XXII and show that in four cases, the CRP was initially negative but had become positive within 24 hours. The ESR was more erratic, with minimal increases in two cases (who died) and a decrease in another. In three cases who survived, there was a brisk increase in the leucocyte count.

Table XXII - Sequential values of several tests in seven infants with proven sepsis.

Case No.	B.Wt. (g)	Sex	Day of +ve SWU	Organism	Initial Values				Values 16-24 hrs. Later				Survived or Died
					WBC	I/T Ratio	ESR	CRP	WBC	I/T Ratio	ESR	CRP	
24	2360	F	1	GBS	3.4	0.58	2	-	8.4	0.38	3	+	D
26	3000	M	5	GBS	6.7	0.30	22	-	25.6	0.21	12	+	S
29	2960	F	0	GBS	5.5	0.48	2	+	23.5	0.30	17	+	S
31	2460	M	0	GBS	11.3	0.33	1	-	13.7	0.49	6	+	D
33	2800	M	1	C.J.	10.4	0.58	10	+	11.7	0.05	38	+	S
39	1080	M	0	E. coli	4.6	0.06	10	+	6.9	0	22	+	S
40	1370	F	6	E. coli	3.5	0.37	2	-	15.1	0.08	15	+	S

GBS = Group B β-haemolytic streptococcus

C.J. = Campylobacter jejuni

SWU = Sepsis work-up

Possible Amniotic Fluid Infection

Among the infants who are more likely to receive antibiotics unnecessarily are those evaluated for possible amniotic fluid infection. Of a total of 276 babies investigated for prolonged rupture of membranes (> 24 hours), maternal fever/infection, and/or unexplained preterm labor, only 6% proved to have sepsis. Of 150 babies evaluated for a single risk factor, only 2 proved to have sepsis, compared to 13 out of 126 babies with multiple factors for investigation ($\chi^2 = 10.93$, $p < 0.001$). The results of the diagnostic tests in this sub-population evaluated for possible amniotic fluid infection are presented in Table XXIII.

Table XXIII - Frequency, predictive value and efficiency of several diagnostic tests used to detect neonatal infection in babies born following PROM (> 24 hours), maternal fever/infection or unexplained preterm labor.

		WBC $<5.0 \times 10^9/\ell$	I/T Ratio ≥ 0.2	WBC < 5.0 and/or I/T Ratio ≥ 0.2	WBC < 5.0 and I/T Ratio ≥ 0.2	Latex CRP +ve	Latex Hp +ve	ESR ≥ 15 mm/h	Sepsis Screen +ve
Total	(n = 276)	20	66	76	10	39	13	8	39
Proven Sepsis	(n = 15)	7	14	15	6	6	4	1	14
Very Probable	(n = 18)	4	15	16	3	13	1	5	15
Sensitivity (%)	- sepsis	47	93	100	40	40	27	7	93
	- infection$^{\Delta}$	33	88	94	27	58	15	18	88
Specificity (%)	- sepsis*	95	80	77	96	87	97	97	90
	- infection$^{\Delta}$	96	85	81	99	92	97	99	96
Positive Predictive Accuracy(%)	- sepsis	35	21	20	60	15	31	13	36
	- infection$^{\Delta}$	55	44	41	90	49	38	75	74
Efficiency (%)	- sepsis	92	81	82	95	85	93	92	91
	- infection$^{\Delta}$	89	85	87	91	88	87	89	95

I/T = Immature/Total Neutrophils

CRP = C-reactive protein

Hp = Haptoglobin

Δ = Proven sepsis and "very probable" infection

* = "Very probable" included as not infected

PROM = Prolonged rupture of membranes

Antibiotic Use

Because the ultimate purpose of using these diagnostic tests was to decrease the indiscriminate use of antibiotics, the use of antibiotics was evaluated before and after the time when the tests were brought to the bedside. Thus, Group 1 consists of babies admitted between October 1975 and December 1977, and Group 2 consists of babies admitted between January 1978 and March 1980.

Details of the composition of the two groups are provided in Table XXIV, and although some differences were noted, it was difficult to predict how they would influence antibiotic use. An increase in the percentage of low birth weight babies in Group 2 was countered by a decrease in the number of males. The change in antibiotic usage is documented in Table XXV, showing a highly significant increase in the number of babies not started on antibiotics. In order to demonstrate that this decrease in antibiotic use was not the result of a change in the reasons for investigation, Table XXVI shows the number of babies investigated for the most frequent presenting features. The number of babies who did not receive antibiotics in the categories considered "not infected" increased for almost every reason for investigation.

Table XXIV - Composition of two groups of babies investigated for sepsis, by sex, weight, gestational age and day of evaluation.

	Group 1 (n = 284)	Group 2 (n = 240)	Chi Square	p Value
Males	173 (61%)	125 (52%)	4.14	< 0.05
Females	111 (39%)	115 (48%)		
Low Birth Weight (< 2500 grams)	169 (60%)	167 (70%)	5.74	< 0.025
Preterm (< 37 weeks)	167 (60%)	169 (70%)	7.63	< 0.01
Evaluated at Birth	147 (52%)	149 (62%)		
" Day 1	68 (24%)	42 (18%)		
" Day 2	29 (10%)	11 (5%)	(3) 8.45	< 0.05
" Day 3-4	29 (10%)	21 (9%)		
" Day 5-7	11 (4%)	17 (7%)		

Table XXV - Antibiotic usage in the two groups, showing the large increase in the percentage of infants not treated with antibiotics

	Group 1 (n = 284)	Group 2 (n = 240)	p Value
Proven Bacterial Sepsis	22 (7.7%)	19 (7.9%)	n.s.
Survival with Sepsis	14 (64.0%)	17 (89.0%)	n.s.
Proven Viral Infection	4 (1.4%)	3 (1.3%)	n.s.
"Very Probable" Infection	12 (4.2%)	22 (9.2%)	n.s.
Positive "Sepsis Screen"	47 (17.0%)	51 (21.0%)	n.s.
Duration of Antibiotics:			
None	39 (14.0%)	113 (47.0%)	
1-3 days	134 (47.0%)	65 (27.0%)	
4-5 days	73 (26.0%)	16 (7.0%)	< 0.0001
> 5 days	38 (13.0%)	46 (19.0%)	
Not Treated with Negative Sepsis Screen	38/237 (16.0%)	111/189 (59.0%)	< 0.0001

Table XXVI - Antibiotic use with the most frequent presenting features, showing a marked increase in Group 2 in the number of babies not receiving antibiotics when those with proven bacterial or viral infection were excluded

	Group 1 (n = 284)		Group 2 (n = 240)	
Risk/Clinical Factors	Number "Not Infected"	Number Not Receiving Antibiotics	Number "Not Infected"	Number Not Receiving Antibiotics
PROM alone	24	3 (13%)	40	30 (75%)
PROM and other	41	5 (12%)	40	18 (45%)
Maternal Infection alone	11	2 (18%)	6	4 (67%)
Maternal Infection and other	24	3 (13%)	20	5 (25%)
Premature Labour alone	27	7 (26%)	43	30 (70%)
Premature Labour and other	18	3 (17%)	21	14 (67%)
Lethargy alone	7	0 (0%)	6	4 (67%)
Lethargy and other	31	2 (7%)	18	8 (44%)
Temp. Instability alone	4	0 (0%)	2	0 (0%)
Temp. Instability and other	13	2 (15%)	8	2 (25%)
Apnoea alone	16	3 (19%)	17	8 (47%)
Apnoea and other	23	3 (13%)	16	4 (25%)
Cyanotic Spells alone	21	1 (5%)	13	5 (38%)
Cyanotic Spells and other	21	3 (15%)	8	3 (38%)

Relationship of Acute Phase Response to Survival

Because it was noted that some babies with leucopenia (and neutropenia) died before demonstrating any increase in C-reactive protein, haptoglobin, α_1-acid glycoprotein or ESR, an evaluation of survival statistics was performed. C-reactive protein and α_1-acid glycoprotein were chosen because they seemed to be more rapid responders than other acute phase proteins.

Since α_1-acid glycoprotein measurements were only performed for Group 1 patients, the analysis is confined to that group. The results are presented in Tables XXVII and XXVIII.

Table XXVII - Outcome in babies with neonatal sepsis related to the presence or absence of a positive latex C-reactive protein test

Latex CRP	Survived	Died	Mean Birth Weight (g)
-ve	3	7	1789
+ve	11	1	2423

Table XXVIII - Outcome in babies with neonatal sepsis related to the level of α_1-acid glycoprotein (orosomucoid)

α_1-AGP (g/ℓ)	Survived	Died	Mean Birth Weight (g)
< 0.5	2	8	1780
> 0.5	12	0	2431

DISCUSSION

> "... the major problem in neonatal infections is the identification of the infected infant. Often overlooked is the equally important task of identifying the non-infected infant. It is desirable to administer appropriate therapy as early as possible to the infected infant, and to avoid such therapy in the others.
>
> (Fulginiti, 1970[63])

> "The emphasis in clinical diagnosis must surely be on very early detection."
>
> (Davies, 1971[3])

Despite the introduction of antibiotics approximately 40 years ago, the infant with neonatal bacterial infection (particularly with sepsis and meningitis) remains at great risk of dying. Survival in this series was approximately 75% when infection was detected in the first week after birth. The mortality rate of 25% is quite comparable to other recent data from Johns Hopkins[64] and Yale[65] and compares to a rate of greater than 90% in the pre-antibiotic era.[66]

One of the major difficulties in making an early diagnosis of infection (particularly sepsis) is the great diversity of clinical manifestations which may suggest infection. Increased awareness of the array of signs which suggest sepsis has led to the investigation of a very large number of babies who are admitted to intensive care (or special care) nurseries. The majority of such babies do not subsequently prove to have infection, but are treated for variable lengths of time with antibiotics.[9,67]

Although positive blood and/or CSF cultures allow a definite diagnosis to be made, a number of other situations make a diagnosis of infection "very probable." This is particularly true when the chest radiograph suggests pneumonia (often indistinguishable from RDS[12-14]) and the tracheal aspirate culture is positive.[68]

The widespread use of antibiotics under many different circumstances has resulted in the emergence of micro-organisms which are resistant to the antibiotics created to kill them.[5,10,11] In the individual case, such considerations are not usually taken into account. Although efforts have been directed at producing new and better antibiotics, Davies has pointed out[69] just how futile this can be, without judicious control of their application.

Among the factors contributing to the widespread use of antibiotics in the intensive care nurseries has been our inability to make a diagnosis of sepsis with any degree of certainty, until blood (or CSF) cultures prove to be positive. One can only reiterate the statement made elsewhere[5] that we need an infallible test (or combination of tests) to make an early diagnosis. Until very recently, laboratory tests have been considered unhelpful or unreliable in making a diagnosis of neonatal sepsis. Two recent chapters in textbooks devoted to infectious disease in children[70,71] make no mention of the possible value of acute phase reactants, although ESR is mentioned in one of them.[71] The present study was conducted to evaluate a variety of tests, either singly or in combination. Although no <u>infallible</u> test (or combination) was found, the leucocyte count and several acute phase proteins provide useful adjunctive information for the clinician trying to make an early diagnosis of neonatal sepsis.

Galen and Gambino have suggested that if a disease is serious but treatable, then the major considerations of a test (or combination of tests) are sensitivity and efficiency.[61] They also suggest that high positive predictive value is essential if treatment of a false positive might have serious consequences.[61] For the individual baby, the administration of antibiotics is unlikely to have serious consequences (although we should not forget the problems associated with chloramphenicol,[72] tetracycline[73] and long-acting sulphonamides[74]). However, for babies in general, indiscriminate antibiotic use can lead to emergence of resistant organisms, which could potentially have lethal consequences.[5,10,11]

A review of the results suggests that some tests are individually very helpful, but certain combinations are more helpful.

Leucocyte Count and Differential

Although a number of authors attested to the value of the leucocyte count in supporting a diagnosis of neonatal sepsis earlier this century,[75,76] it was generally dismissed as being an unreliable test because of its great variability.[77,78] A little over a decade ago, interest in the value of the leucocyte count was rekindled by the work of Xanthou[17,79] and others.[18,19]

Since that time, a considerable body of information has been published on several aspects of the leucocyte count, such as total leucocyte count,[16,22] absolute neutrophil count,[18,19] band (immature) neutrophil count,[20,21,23] morphology of neutrophils[20,21] and different neutrophil ratios.[16,23-25,28] However, as late as

1974 (just before this study began), the leucocyte count was considered to be "usually unrevealing or uninterpretable," in a major review of neonatal sepsis.[78] Even five years later, another review stated that "peripheral white blood cell counts are generally not reliable in the diagnosis of newborn sepsis."[4] Other authors claimed that "when properly interpreted, (it) may aid in the early recognition of neonatal infection."[25] The present study supports the claim of these latter authors, even without making allowance for confounding variables. For example, it has been shown that maternal hypertension, maternal fever, periventricular haemorrhage and asphyxia[25,27] can influence neutrophil counts, and lower neutrophil counts were associated with lower gestational ages in one study.[26] Although decreased birth weight (and gestational age) was associated with lower total leucocyte and neutrophil counts in non-infected babies in this study, the immature/total neutrophil ratio was not affected. A number of non-infected very low birth weight infants had very low total leucocyte counts on the first day (see Figure 1). The lower limit of $5.0 \times 10^9/\ell$ was chosen somewhat arbitrarily, but has been used frequently by others.[29,77] It was not apparent that a lower limit (e.g., $4.0 \times 10^9/\ell$[2,53]) could improve the predictive value of the test, without losing something in sensitivity.

The single most useful test appears to be the immature/total (I/T) neutrophil ratio. This index of infection was first described by Manroe and her colleagues,[23] although similar indices have been described by others.[16,24,53] The upper limit of normal seems to be 0.16 on the first day, so that using equal to or greater than 0.2 seems reasonable (at least on the first day). Subsequently, use of

0.15 or greater might be more appropriate. However, it seems simpler to use a single number at all ages, and it is easy to remember in conjunction with wbc $< 5.0 \times 10^9/\ell$ (since 0.2 = 1/5 th).

Although not very sensitive, the combination of both wbc < 5.0 and I/T ratio ≥ 0.2 was very predictive of sepsis (and infection). Of 22 infants in the first week with this combination (see Table XX), 15 (68%) proved to have sepsis and 19 (86%) proved to have "infection." When either one or other of these tests (wbc < 5.0 and/or I/T ratio ≥ 0.2) was used, sensitivity improved greatly, so that 38/41 (93%) babies with sepsis were detected, but with accompanying loss of predictiveness (positive predictive value = 23%). Efficiency was less than 80% (see Table XIII).

It has been suggested[22,80] that falling leucocyte counts in the first 24 hours after birth may be more predictive of sepsis than single determinations. This hypothesis could not be adequately answered by this study since repeat determinations were not performed routinely, but isolated examples of this phenomenon were noted. Another feature not routinely evaluated was abnormal morphology of the neutrophils. Although Zipursky and his colleagues drew attention to abnormalities associated with neonatal infection,[20,21] more recent work casts some doubt on the usefulness of such evaluation.[14]

The major advantage of the leucocyte count is its ready availability in any hospital. By using the I/T ratio ≥ 0.2 alone, most infants with infection will be detected. The addition of wbc $< 5.0 \times 10^9/\ell$ provides support that sepsis is present. Extremely elevated I/T ratios (> 0.80) seems to carry a very poor prognosis.[28]

Erythrocyte Sedimentation Rate

For many years the erythrocyte sedimentation rate was considered somewhat impractical in neonates because it required a blood sample of 2-3 ml. There was also doubt about its value in this age group. In 1970 Evans et al.[32] "rediscovered" a microtechnique using a specially designed pipette. However, this technique was not widely used. More recently, Adler and Denton described a very simple technique using a microhematocrit capillary tube.[33] The volume of blood required is approximately 75 µℓ.

This new technique makes it very easy to perform an ESR, but there is little critical appraisal of its value in assessment of the neonate. Two other small studies[62,81] using this technique, have shown similar values to those originally published for non-infected babies and support the contention that ESR is helpful in diagnosing infection. In the present study, it is apparent that there is frequently a delay in the elevation of ESR in infected infants (as previously suggested[33,47]). However, when the ESR is elevated, it is quite predictive (69%) of infection.

Thus, the absence of an elevated ESR cannot be used to exclude infection, but when the ESR is equal to or greater than 15 mm/h, it is strongly suggestive that infection is present, particularly after the first day.

Although it is generally believed that the major determinant of ESR is an elevated fibrinogen level, one might question this in the neonate. According to Relier et al., fibrinogen is a quite rapidly responsive acute phase protein and is usually elevated in infants

with infection,[52,54] but a recent report suggests that elevated fibrinogen levels may be seen in other sick infants who are not infected.[82] However, an assessment of the relationship of fibrinogen to ESR has not been made in the neonate. One confounding variable in neonatal sepsis is the possibility of disseminated intravascular coagulation.[83] Since fibrinogen levels are markedly depressed with DIC, it is unlikely that the ESR will be elevated under these circumstances. This should not have had a significant impact on the results of this study, since DIC is unlikely to occur early in the course of infection. Further support for this contention is the fact that thrombocytopenia was quite uncommon.

C-reactive Protein

The discovery of the precipitation reaction between pneumococcal C carbohydrate and acute phase human sera gave rise to the name C-reactive protein (CRP).[84] Since that time, much has been learnt about the structure and function of CRP,[85-87] although comparatively little is known about the kinetics of this protein in the neonate. One of the attractive features of this acute phase protein is that it is usually present in very small amounts in "normal" individuals (both adult and newborn), so that its presence in more than small amounts is indicative of inflammation.[85-87] It appears to be one of the most rapidly responsive acute phase proteins, with significantly elevated levels appearing within six hours of the induction of inflammation.[87] It also rises to levels which may be 100 to 1000 times those found under normal circumstances.[87]

Although in older individuals other inflammatory reactions (e.g., rheumatoid arthritis[88]) may produce elevated CRP levels, inflammation in the neonate is almost exclusively limited to those with infection. Despite earlier reports of elevated levels,[89,90] the most useful paper (prior to the start of this study) documenting increased CRP in neonatal infection was by Sabel and Hanson.[47] More recently, Sabel and Wadsworth have documented the frequency with which CRP is increased in infants with neonatal sepsis, using a modification of the latex CRP method,[48] and a study from Spain[91] supports their conclusion that CRP is frequently increased in neonatal sepsis. One of the problems with earlier studies of CRP was the time taken to obtain a result. The most satisfactory quantitative technique was radial immunodiffusion, but this technique requires 18 to 24 hours to obtain a result. The major advantage with the latex CRP technique is its rapidity, since an answer can be obtained in ten minutes. As usually performed, the limitation of the test is that it is semi-quantitative (a positive test is obtained with levels greater than 8 mg/ℓ). In this study, a positive latex CRP test was one of the more sensitive tests for infection and was reasonably efficient. This was particularly true in the older infants. Although not evaluated routinely, some infants with sepsis, with negative CRP tests when first investigated, had positive tests within 12-24 hours (see Table XXII). However, this study was designed to find <u>early</u> diagnostic tests, rather than determining how frequently the tests became positive at <u>some</u> time in the course of investigation.

Haptoglobin

Haptoglobin, which was discovered by Polonovski and Jayle,[92] is so named because it binds free haemoglobin released into plasma from red cell breakdown. The haemoglobin-haptoglobin complex is removed by the reticulo-endothelial system. In addition to this function, haptoglobin was also found to be an acute phase reactant.[93]

For many years it was considered that the majority of neonates did not have demonstrable haptoglobin, at least in the first few days after birth. However, although the levels are considerably lower than in adults, haptoglobin can be demonstrated in almost all neonates.[46,94-96] Thus, the fact that the haptoglobin level in most neonates is low means that infection is probable when haptoglobin levels are increased.[46]

In the present study, it appears that haptoglobin is slower to respond than CRP. Although not very sensitive alone, it is quite predictive of sepsis. The latex haptoglobin test was positive in several infants with sepsis who had negative latex CRP tests.

α_1-Acid Glycoprotein (Orosomucoid)

Although other acute phase proteins (apart from fibrinogen, CRP and haptoglobin) are available for study, the only one which has attracted limited attention for diagnosis of infection is α_1-acid glycoprotein. This was first described in the neonate in 1971 and published in 1973.[49] Subsequent studies in neonatal infection have been published in Japanese,[97] French[50] and German,[98] with varying degrees of enthusiasm. In a recent report in English, Sann and colleagues have provided the most extensive experience with this

acute phase protein.[51] Their experience is confirmed in the present study. In general, α_1-acid glycoprotein seems to be a useful diagnostic aid in neonatal infection. Extremely elevated levels have been found in some infected infants, early in the course of infection. On the other hand, some infants with sepsis fail to produce substantial amounts of this protein,[51] which may have poor prognostic significance (see Table XXVII).

In this study, radial immunodiffusion was used to measure α_1-acid glycoprotein. As mentioned previously, this method requires 18-24 hours to obtain a result. This clearly limits its usefulness in diagnosis. However, the recently described experience using laser nephelometry (which can provide a result within two hours or less) appears to offer an attractive alternative.[51] Although not entirely clear, α_1-acid glycoprotein may be more rapidly responsive than haptoglobin. Further studies are required to decide whether or not α_1-acid glycoprotein could (or should) replace haptoglobin.

The levels which may be considered as the upper limits of normal are 0.5 g/ℓ on the first day and 0.75 g/ℓ thereafter. However, it may be important to consider gestational age in the interpretation, since there seems to be a gradual increase in levels in the last trimester of gestation. In addition to the diagnostic use of α_1-acid glycoprotein, it might also prove to be valuable in management (i.e., decisions about discontinuing antibiotics).[51]

In the preterm baby who presents with respiratory difficulty, it may be helpful to use an α_1-acid glycoprotein level to distinguish between respiratory distress syndrome (RDS) and pneumonia. Low levels have been reported in cord blood of babies who subsequently develop RDS,[99] and increased levels were noted with "congenital" pneumonia.[49] However, more recent evidence suggests that 14% of patients with RDS may have initially high levels.[51]

Tests of Limited Value

As noted in the Results, several tests initially considered as potentially useful seemed to be of limited value.

Platelet Count - This indicant does not seem to be useful in making an early diagnosis. Previous reports have indicated that this is a useful marker of neonatal infection.[30,31] It is true that when all infants with sepsis are evaluated, many of them will have thrombocytopenia at some point in their illness. However, this study supports the findings of other recent studies,[13,16,22,24,26] which indicate that thrombocytopenia is a late feature of neonatal sepsis.

Immunoglobulin M - After an initial wave of enthusiasm for the potential value of IgM levels in neonatal sepsis,[44,45,100] there has been little support for this test as an early indicator of neonatal sepsis in the past decade.[101] IgM cannot be considered an acute phase reactant, since it takes approximately 2-3 days (or more) before any response to

infection is seen. This lack of response was noted (among others) by Salmi.[46] The increased levels which may be seen after a few days may take three or four weeks to drop.[102]

Gastric Aspirate Smear - The presence of five or more polymorphonuclear leucocytes per high-power field has been proposed as a useful indicator of infection.[34] However, it was subsequently shown that the source of the leucocytes was maternal, not fetal.[103] This means that it may provide a better indication of risk of infection (or exposure), but does not necessarily indicate infection in the neonate. Others have questioned the value of this test[4,35,104] and the present results also suggest that this is a test of limited usefulness.

Tests Not Evaluated

A number of other tests proposed as helpful in the diagnosis of neonatal infection were not evaluated, for several different reasons. Among the more important of them were the following:

Nitroblue Tetrazolium Reduction Test - At the time this study was initiated, there was considerable debate about the reliability of the NBT test in the neonate.[39] Subsequently, it has been suggested that careful standardisation of the amount of heparin used can eliminate some of the problems.[40]

Recently it has been proposed that the NBT test (with a new method) is a good screening test for infection.[105] It should be pointed out that the test cannot be performed in the presence of neutropenia, and does not clearly differentiate babies with sepsis (and respiratory distress) from those with RDS (hyaline membrane disease).[13]

Buffy-Coat Smear - This test was proposed for use in the neonatal period after the initiation of the study.[37] In fact, it was used in a few babies (after the report was published), with several positive results. However, it suffers from the same drawback as the NBT test, in that a buffy coat smear cannot be evaluated with leucopenia and/or neutropenia. It is necessary to be able to see bacteria engulfed by the neutrophils. It is also recommended that statements regarding a negative test should not be made until a search has been pursued for half an hour (or the whole slide carefully examined). These limitations mean that (from a practical point of view) a positive test is helpful,[13,37] but a negative test must be interpreted with caution, and many times the test cannot be interpreted (because of neutropenia). Even a positive test may require cautious interpretation, if findings in adults[106] are duplicated in the neonate. A more recently described variation of this technique indicates that direct blood smear for bacteria may provide equally reliable information.[107] However, the same time limitations apply.

Cord Histology - Another test which was initially promoted as being useful to diagnose "amniotic fluid infection" was histological examination of the umbilical cord.[41,42] There are limitations of time, availability, practicality and interpretation,[43,104] which make this test of limited value in evaluating sepsis throughout the neonatal period. A variation, which has not been widely used, is the bacteriological examination of a placental smear.[52,108]

Combination of Tests

The majority of studies which describe the value of diagnostic tests for neonatal sepsis concentrate on a single test. Usually, retrospective analysis has been performed on babies with sepsis, to focus on a test which was frequently positive. In some studies, it is quite unclear when the test being evaluated was performed, in relation to the onset of the illness. In this study, all tests were performed at the time that sepsis was suspected and cultures were sent. This allows one to evaluate the usefulness of each test, under the clinical circumstances usually encountered.

Although some authors[13,53,97] have looked at several tests when trying to designate babies as infected or non-infected, there are very few studies where an attempt has been made to look at combinations of tests. One of these was published in Japanese[96] and described an acute phase reactants score, which used the combination of haptoglobin, C-reactive protein and α_1-acid glycoprotein (orosomucoid). Although described as a screening test,

the technique of measurement of the proteins was radial immunodiffusion. As mentioned previously, a minimum of 18 to 24 hours is required to obtain reliable results. Such a delay does not allow the results to be incorporated into clinical decisions about antibiotics. In particular, many babies with early-onset infections may die within 18 to 24 hours (with or without treatment), so that decisions must be made as soon as possible. Another scoring system has recently been described from Mexico, combining CRP, α_1-antitrypsin, orosomucoid, ESR and platelets.[109] After evaluating 56 patients, they concluded that platelet count and ESR were enough to make the diagnosis of sepsis.

The present scoring system is the only other combination of tests of which I am aware (although some authors have combined clinical features and parts of the leucocyte count[29,53]). This combination of tests may be considered a "sepsis screen" in the first few days after birth, when many babies are investigated, but few are infected. Later in the neonatal period, the tests are more confirmatory than screening, since a much higher percentage of babies evaluated proved to have sepsis. The combination of acute phase proteins with the leucocyte count seems to provide a more reliable assessment than either alone. When the sensitivity, positive predictive accuracy and efficiency of the tests is considered (Table XIII), it is clear that the combination of two or more of the five tests is superior to any individual test. Whether or not even greater sensitivity or predictiveness could be achieved by substituting other tests (e.g., α_1-acid glycoprotein for haptoglobin, fibrinogen for ESR) remains to be seen.

It may be important to remember that the predominant organisms in this study were group B streptococcus and Escherichia coli, but a variety of organisms was represented. Nevertheless, the combination may not be as sensitive or predictive when other organisms are predominant. For instance, a recent report described non-group D alpha-hemolytic streptococcus as a common pathogen in neonatal sepsis and showed only 1 of 31 septic babies with wbc < $5.0 \times 10^9/\ell$.[110] In another report on the value of C-reactive protein, two neonates with listeria monocytogenes infection failed to increase CRP levels.[47] In view of the fact that other organisms may be more prevalent in other countries (e.g., listeria monocytogenes in France,[111] staphylococcus epidermidis in England,[112] staphylococcus aureus in Sweden[113] and salmonella enteritidis in Saudi Arabia[114]), it may be necessary to re-assess this combination of tests in other centers.

Bedside Use of Tests and Antibiotic Decisions

As noted previously, the difficulty in reaching a definite diagnosis of neonatal sepsis has resulted in a rather indiscriminate policy of antibiotic administration. Most babies who are suspected of having sepsis have antibiotics started after cultures are taken.[4,5,9] In those situations where the primary reason for investigation was a single "risk" factor, the yield of sepsis was very low, in this study.

When the combination of tests referred to as a "sepsis screen" was brought to the bedside in the second half of the study, there was a marked decrease in the number of babies placed on antibiotics. This could not be accounted for on the basis of a significant change

in the reasons for investigation, and a reduction in antibiotic administration was noted for each of the main reasons for doing a "sepsis work-up." Although low birth weight infants are more susceptible to infection,[2,5,115] the same can be said of males.[3,116,117] Thus, the marked change in antibiotics use could not be ascribed to the decrease in the percentage of male infants, which was offset by the increase in the percentage of low birth weight infants.

Concomitant with the decreased administration of antibiotics was an improved survival rate. It therefore appears that the "sepsis screen" was a useful adjunct in making a diagnosis of sepsis, without increasing the risk of overlooking babies who were infected but had negative tests. In this study, the ratio of babies treated with antibiotics to babies with proven sepsis fell from 11:1 to 6.6:1, which compares favorably to ratios of 15:1 and 28:1 in two Boston hospitals.[118]

The policy of administering antibiotics to the majority of infants who are suspected of having sepsis has resulted in the emergence of resistant organisms, which may be very difficult to treat.[5,10,11] Any decrease in the use of antibiotics should counteract this tendency and also minimise the chance of side-effects (e.g., with chloramphenicol). Even when antibiotics are started, persistently negative tests may allow them to be stopped when cultures prove to be negative after 48 to 72 hours. Others have shown that a negative CRP best supports stopping treatment in doubtful cases.[119] By carefully evaluating each clinical

situation and restricting the usual indications for prophylactic therapy, physicians in Paris were able to decrease antibiotic use from approximately 80% of admissions to a special care nursery to approximately 20%.[120] In addition, they noted that the total number of infections did not increase, that Gram negative infections became relatively less frequent and that more sensitive organisms replaced them.[120]

Early Diagnosis of Sepsis

As stated earlier, it seems clear that clinical suspicion of neonatal sepsis is the most important step in arriving at an early diagnosis, but it is frequently difficult on clinical grounds to distinguish sepsis from many other disorders of the newborn infant. This study demonstrates the usefulness of a handful of simple tests, which are relatively inexpensive and can be performed rapidly. The results can be available within an hour, and when used in combination (any two or more of five tests), seem to be both highly sensitive and highly predictive. Babies not detected with this technique were usually very sick, left no doubt in the physician's mind that antibiotic treatment was necessary, and may have been incapable of mounting an appropriate response to infection. These tests provide useful adjunctive information (together with clinical assessment) in making an early diagnosis of sepsis.

In general, we may assume that the earlier a diagnosis is made, the earlier antibiotic treatment will begin, and the more likely that survival will be the outcome. However, some cases of infection are so fulminant (e.g., early-onset group B streptococcal sepsis,[121,122]) that additional forms of treatment, such as exchange transfusion[123,125] or granulocyte transfusion[126,127] may be required. As a result of the newer techniques of countercurrent immunoelectrophoresis[128,129] and latex particle agglutination,[130,131] it may be possible to make a specific diagnosis within a short period of time (usually determined by the baby's ability to produce urine, since this seems to be the most reliable source for detecting antigen.[132,133] Because there are a large number of organisms which are potentially pathogenic for the neonate and a limited number of antisera, the non-specific diagnostic tests described in this study should probably be evaluated first. If a specific diagnosis can be made, antibiotic choice may be guided by knowledge of local sensitivities.

In some referral centers, it is not uncommon to have babies referred who have been started on antibiotics without first taking cultures. Under such circumstances, the tests described here may allow the diagnosis of sepsis or "very probable" infection to be made with greater confidence.

CONCLUSIONS AND IMPLICATIONS

> "... the search for the perfect quick diagnostic combination should go on -- especially on behalf of the hard-pressed house officer responsible for a neonatal intensive care unit in the small hours ..."
>
> (Editorial, Br. Med. J., 1979[5])

While the combination of tests described here cannot be considered infallible, nevertheless these tests seem to provide the most reliable indicators of infection so far described. It will be important to evaluate them in other centers, particularly where other organisms are more prevalent. By combining the leucocyte count with acute phase proteins, one may be "getting the best of both worlds."

The simplicity of the tests and the rapidity with which the results can be obtained mean that they 1) could be available in almost any hospital where babies are delivered, 2) could help to make an early diagnosis of neonatal sepsis, and 3) could be incorporated into decisions about whether or not to start antibiotics. In this investigation, the tests were accepted as valuable by house officers and a decrease in antibiotic use resulted. This was most pronounced when a single "risk" factor (e.g., prolonged rupture of membranes) was the reason for investigation.

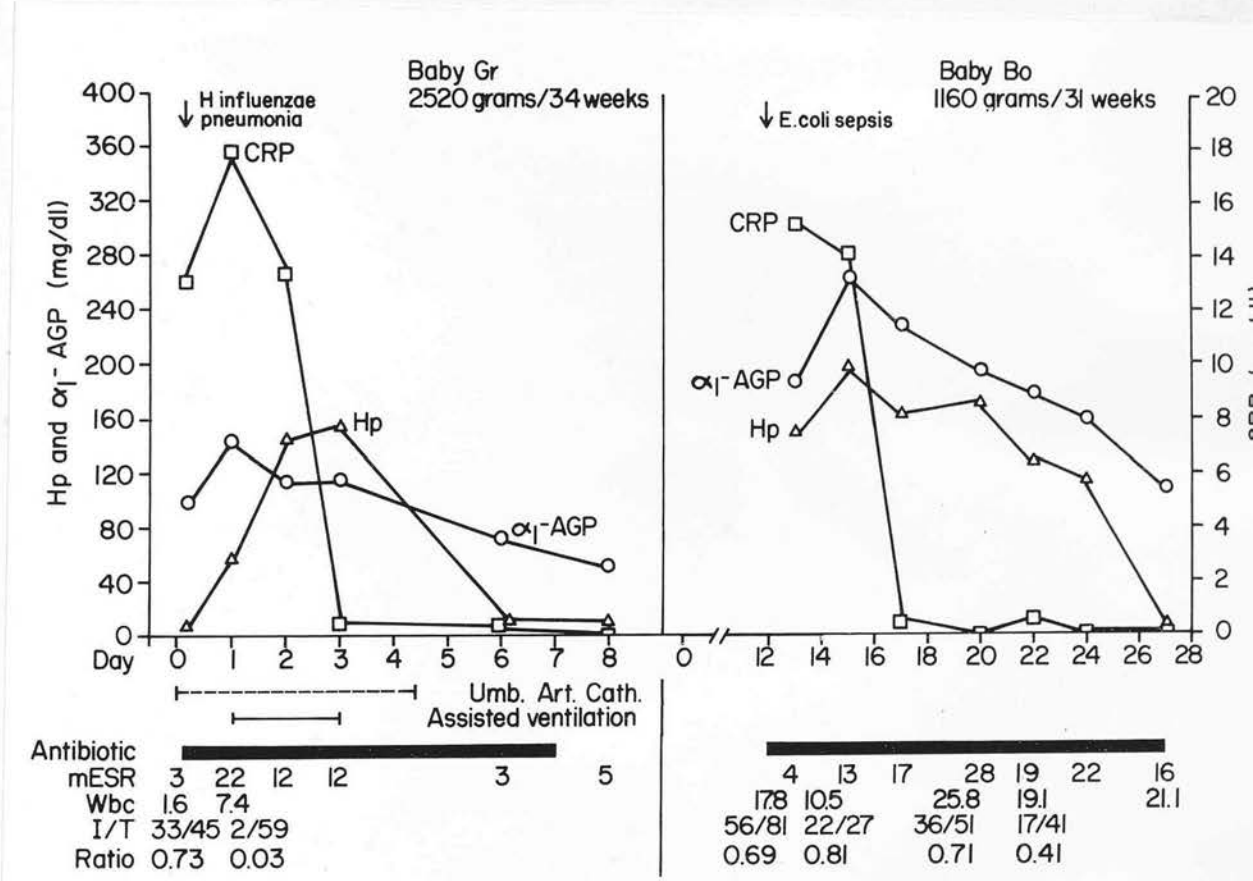
The ability to produce acute phase proteins may provide some protection against infection. There is some information to support the idea that C-reactive protein may enhance phagocytosis[86,87] and may also be incorporated into the complement pathway.[86,87] Additional effects of α_1-acid glycoprotein on lymphocytes have been described,[134]

but the function of acute phase proteins is currently the subject of considerable investigation.[86,87]

Perhaps the most important use of the acute phase proteins will be in following the subsequent course of an illness. Although diagnosis is enhanced, there is usually some lag in response of the acute phase proteins, so that occasionally one might have to rely entirely on the leucocyte count. Since the acute phase proteins may increase later, this might confirm diagnosis. (They may possibly confirm infection in cases where antibiotics are started before cultures are obtained). More importantly, the documentation that levels had returned to normal would provide objective evidence that the antibiotic treatment was effective. In this regard the findings of De Gamarra et al. are very interesting.[54] In studying fibrinogen levels, they noted persistence of high levels in infants with sepsis where the infecting organism was resistant to the antibiotics chosen.[54]

In addition, the work of Sabel and Hanson should not be overlooked.[47] They showed that, in infants with meningitis who had elevated CRP levels at the time antibiotics were stopped, recurrences of infection occurred. This was not the case when CRP levels were within normal limits.[47] The recent work of Sann et al.[51] also attests to the value of following α_1-acid glycoprotein levels sequentially. Some recent (unpublished) work of the author suggests that combining C-reactive protein, haptoglobin and α_1-acid glycoprotein may prove helpful. Two representative examples are provided in Figure 6.

Figure 6 - Quantitative determinations of C-reactive protein (CRP), haptoglobin (Hp) and α_1-acid glycoprotein (α_1-AGP) levels in infants with Hemophilus influenza pneumonia and Escherichia coli sepsis are contrasted with leucocyte and ESR values. (I/T = immature/total neutrophils)



At this point it is not completely clear what the patterns of response of the acute phase proteins will be. It seems probable that C-reactive protein can be used to establish efficacy of treatment (when it falls) and other proteins (e.g., haptoglobin and α_1-acid glycoprotein) can be used to decide when antibiotics can be safely stopped. However, different bacteria may produce different patterns of response. Further studies are clearly indicated.

One other area which requires further investigation relates to the role of endotoxin. Recent evidence indicates that endotoxinaemia in neonates, without positive blood cultures, may be more common than previously thought.[135-137] Since endotoxin is known to produce an acute phase response,[138,139] some of the "false-positives" may prove to be on the basis of endotoxinaemia. Newer microtechniques for measuring endotoxin may answer the question.

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LIST OF ABBREVIATIONS

α_1-AGP	=	α_1-acid glycoprotein
CRP	=	C-reactive protein
CSF	=	cerebrospinal fluid
DIC	=	disseminated intravascular coagulation
E. coli	=	Escherichia coli
ESR	=	erythrocyte sedimentation rate
(mESR	=	mini-ESR using microhematocrit tube)
Hp	=	haptoglobin
I/T ratio	=	immature/total neutrophil ratio
IgM	=	immunoglobulin M
NBT	=	nitroblue tetrazolium reduction test
n.s.	=	not significant
PROM	=	prolonged rupture of membranes
RDS	=	respiratory distress syndrome
SGA	=	small for gestational age
Staph.	=	Staphylococcus
Strep.	=	Streptococcus
SWU	=	sepsis work-up
Temp.	=	Temperature
UTI	=	urinary tract infection
WBC	=	white blood cell count
-ve	=	negative
+ve	=	positive

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THE PROTECTIVE EFFECT OF ACUTE PHASE REACTANTS IN NEONATAL SEPSIS

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ABSTRACT. Philip, A. G. S. (Department of Pediatrics, University of Vermont, USA). The protective effect of acute phase reactants in neonatal sepsis. Acta Paediatr Scand, 68: 481, 1979.—Phase reactants were evaluated prospectively in babies suspected of having sepsis. Among 318 babies, there were 22 proven and 10 "very probable" cases of neonatal sepsis. Of the proven cases 14 survived and 8 died. The survivors had a positive latex C-reactive protein (CRP) in 11 cases and an alpha$_1$-acid glycoprotein (AGP) level greater than 0.5 g/l in 12 cases. Among those who died, one had a positive latex CRP and none had AGP >0.5 g/l. These findings were supported by positive CRP and elevated AGP in almost all "very probable" cases, all of whom survived. These data in newborn infants support the hypothesis that acute phase reactants have a functional role in combating infection.

KEY WORDS: Newborn, sepsis, acute phase reactants

In the presence of inflammation or infection, the concentration of a number of serum proteins increases as the "acute phase response". The evaluation of these "acute phase reactants" in the newborn period has been primarily directed at the diagnosis of infection. Acute phase reactants which have been evaluated in neonatal sepsis include C-reactive protein (8), haptoglobin (9), α_1-acid glycoprotein (orosomucoid) (3, 10) fibrinogen and other proteins which influence the erythrocyte sedimentation rate (1, 7).

Little attention has been paid to the *function* of these acute phase proteins. C-reactive protein has been described as a phagocytosis-promoting factor (4). Recent experimental evidence indicates that in adults both C-reactive protein (5), and α_1-acid glycoprotein (2) may play an important role in modifying lymphocyte responsiveness.

This report provides evidence, in newborn infants, which supports the hypothesis that acute phase reactants may be important in helping to combat infection.

MATERIALS AND METHODS

Acute phase reactants were evaluated in a prospective study of neonatal sepsis during the first week of life (6). Serum was obtained from 318 babies who were being investigated with a "sepsis work-up" for either risk factors (e.g., prolonged rupture of membranes, smelly amniotic fluid, maternal fever, etc.) or clinical manifestations of neonatal sepsis (e.g., lethargy, abdominal distension, temperature instability, etc.).

There were 22 babies with proven bacterial sepsis who provide the main focus of this report. Several diagnostic tests for sepsis were used, including C-reactive protein (CRP) and α_1-acid glycoprotein (AGP).

C-reactive protein was measured with a latex reagent method[1] which provides a positive reaction with levels equal to or greater than 8 mg/l.

Alpha$_1$-acid glycoprotein (orosomucoid) was measured with an immunoduffusion method.[1]

Low birth weight (LBW) is defined as less than 2 500 g.

RESULTS

Of the 22 babies with proven bacterial sepsis, 14 babies survived (7 LBW) and eight babies

[1] Reagents kindly supplied by Behring Diagnostics, Somerville, New Jersey.

Table 1. *Outcome in babies with neonatal sepsis related to the presence or absence of a positive latex C-reactive protein test*

Latex CRP	Survived	Died	Mean birth weight (g)
−ve	3	7	1 789
+ve	11	1	2 423

Table 2. *Outcome in babies with neonatal sepsis related to the level of α_1-acid glycoprotein (orosomucoid)*

α_1 AGP (g/l)	Survived	Died	Mean birth weight (g)
<0.5	2	8	1 780
>0.5	12	0	2 431

died (7 LBW). The babies were classified according to whether they lived or died, and with respect to C-reactive protein (Table 1) and α_1-acid glycoprotein (Table 2). It is clear that when either of these two phase reactants was present in significant amount, the likelihood of survival was very good. When low levels of phase reactant were present, death from infection was likely.

The mean birth weight of the survivors was 2 517 g versus 1 466 g for those who died. However, it is pertinent to point out that one infant with birth weight 1 180 g had a positive CRP and AGP of 1.44 g/l and survived, and another infant with birth weight 2 665 g had a negative CRP and AGP of 0.15 g/l and died of infection. It is also noteworthy that all of 10 infants with clinical features strongly suggestive of sepsis, but without a positive blood culture, survived. Nine had AGP greater than 0.5 g/l and eight had a positive CRP (Table 3).

Evidence that birth weight may not be the major determinant is provided by the following facts. Babies with weights over 2 500 g without evidence of infection ($n=89$), had a mean level of AGP of 0.324 g/l, compared to a mean level of 0.265 g/l for the total group without evidence of infection ($n=281$). There were 33 babies in this latter group who had AGP levels greater than 0.5 g/l. Of these, 12 babies were LBW. CRP was positive in 19 babies without evidence of infection. Eleven of the 19 babies were low birth weight. The smallest baby with a positive test weighed 1 020 g.

Table 3. *Findings in 10 babies who had strong presumptive evidence of infection but lacked a positive blood culture*

S.W.U. = "sepsis work-up". G.B.S. = Group B Beta-hemolytic streptococcus

Identity no.	Age at S.W.U.	Birth weight (g)	Sex	CRP	α_1AGP (g/l)	Factors suggesting infection
1/16	2 d.	3 544	M	+ve	0.64	Pneumonia
1/41	8 h	2 041	M	−ve	0.84	W.B.C. = 2 300/mm^3; pneumonia; maternal G.B.S.
1/104	12 h	2 840	M	+ve	0.39	Lethargy; tracheal aspirate—G.B.S.
1/112	2 d.	4 111	M	+ve	0.66	Fever, pustule grew staph. aureus coag. positive
1/166	4 d.	2 280	M	−ve	0.67	Poor feeding; shock; W.B.C = 3 200/mm^3
2/40	2 d.	2 060	F	+ve	0.58	Pneumonia; tracheal aspirate—G.B.S.
2/46	4 h	2 980	F	+ve	0.93	Apnea; shock; double dose antibiotics I.V. before transfer and before blood culture
2/99	6 h	3 232	F	+ve	0.91	Fever; maternal G.B.S.
2/114	4 h	4 000	M	+ve	0.96	Maternal fever and antibiotics; tracheal aspirate—E. coli; antibiotics I.V. before transfer and before blood culture
2/120	3 d.	2 395	F	+ve	>2.0	Pneumonia; fever

DISCUSSION

Evaluation of babies with proven bacterial sepsis showed that elevated levels of two proteins considered to be "acute phase reactants" were strongly correlated with mortality. Those newborn infants who had elevated levels of C-reactive protein and α_1-acid glycoprotein, when sepsis was first suspected, survived. Those with low levels of CRP and AGP usually died. These findings strongly support the hypothesis that both CRP and AGP play an important role in the ability to combat infection.

Although there appears to be a strong influence of birth weight upon survival or death from neonatal sepsis, there were several notable exceptions. It seems more likely that the very premature (preterm) newborn infant has difficulty with, or is sometimes incapable of, mounting an appropriate response to bacterial infection. When a baby is able to respond appropriately by producing (increasing) acute phase reactants, there appears to be a protective effect.

The findings in the cases of proven bacterial sepsis support the hypothesis of a protective effect of acute phase reactants. Further support is provided by the elevated levels of CRP and AGP found in the cases of very probable infection, all of whom survived. Elevated levels are infrequently seen when there is no evidence of infection. The values for CRP indicate that a positive response is not determined entirely by birth weight. Less than 10% of babies weighing more than 2 500 g had a positive test, and several very LBW babies were able to produce an increase in CRP. The mean level for AGP in heavier babies was not dissimilar to the total group.

The function of acute phase reactants has been speculative until recently. Evidence in vitro has indicated the importance of CRP and AGP in "modulating lymphocytes" (2, 5). CRP increased in vitro phagocytosis of *D. pneumoniae*, *Staph. aureus*, *E. coli* and *Klebsiella aerogenes* (4). The evidence presented here strongly suggests that in newborn infants acute phase reactants also have a protective effect against neonatal sepsis.

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Early Diagnosis of Neonatal Sepsis

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ABSTRACT. To better define the need for antibiotic therapy, several tests recommended as helpful in diagnosing neonatal sepsis were evaluated in 376 neonates during the first week after birth. The five most useful tests (with definitions of abnormality) were: band/total neutrophils (≥0.2); leukocyte count (<5,000/cu mm); latex-C-reactive protein (positive >0.8 mg/100 ml); ESR (≥15 mm for the first hour); and latex haptoglobin (positive >25 mg/100 ml). When these five tests were applied early (at the time infection was suspected and blood culture sent), 28 of 30 cases (93%) subsequently proven to have infection had two or more abnormal tests. This compares with only 24 of 320 babies (8%) with no subsequently documented evidence of infection. Of all babies who had two or more tests positive (n = 71), 39% had proven sepsis, and an additional 23% had "very probable" infection. The combination of leukopenia and an elevated band/total neutrophil ratio seems to be particularly predictive of sepsis (13 of 17 babies with this combination had proven sepsis). When less than two tests were positive, the probability that sepsis was *not* present was 99%. These simple, rapid tests require no special laboratory facilities and provide a valuable adjunct in the early detection of the neonate with sepsis. *Pediatrics* 65:1036–1041, 1980; *newborn, sepsis, infection diagnosis, acute phase reactants, leukocytes.*

The major problem in neonatal infections is the identification of the infected infant. Often overlooked is the equally important task of identifying the noninfected infant. It is desirable to administer appropriate therapy as early as possible to the infected infant, and to avoid such therapy in the others.[1]

Diagnosis of neonatal septicemia is one of the most difficult tasks in clinical medicine.[2]

A number of independent observers have suggested that several different laboratory determinations are individually helpful in detecting bacterial infection in the newborn infant. These include serum immunoglobulin M,[3,4] neutrophil and band counts,[5–7] C-reactive protein[8,9] serum orosomucoid (α_1-acid glycoprotein),[10,11] serum haptoglobin,[12] serum fibrinogen,[13] erythrocyte sedimentation rate,[14,15] buffy-coat smears[16] and immature/total neutrophil ratio.[17] The difficulty in making an early diagnosis of neonatal sepsis, despite improved bacteriologic techniques, is attested to by recent reviews.[2,18,19]

Therefore, a group of tests was studied to assess their usefulness, either singly or in combination, in predicting neonatal sepsis. The major objective was to establish a rapid assessment which would provide a reliable early identification of neonates with bacterial sepsis.

MATERIALS AND METHODS

Babies included in the study were admitted to the intensive care nursery at the Medical Center Hospital of Vermont between October 1975 and June 1979. Any baby suspected on clinical grounds of having sepsis or meningitis in the first week after birth was included in the study. Certain high-risk categories (prolonged rupture of membranes, maternal fever, premature labor without good reason, etc) and certain clinical situations (abdominal distension, lethargy, temperature instability, etc) have been well documented previously[1,2,18–20] and provided the basis for initiating investigation for systemic infection.

When a newborn with suspected sepsis (or meningitis) was identified, evaluation included a gastric aspirate for smear when indicated (positive when more than five polymorphonuclear leukocytes per high power field were seen),[21] a white blood cell count and differential, platelet estimate, and blood, urine, and cerebrospinal fluid cultures (the last two were occasionally deferred). Blood was sent for both aerobic and anaerobic cultures. Samples were sent for viral cultures in any case where viral infection

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seemed likely. When a baby had associated respiratory signs (tachypnea or dyspnea) a chest radiograph was obtained.

After obtaining permission from a parent, an extra sample of blood (0.5 to 1 ml) was taken for the following studies: (1) immunoglobulin M (IgM), performed by the gel immunodiffusion method, and with a rapid latex method[22] (2) C-reactive protein (CRP) with the latex method; (3) α_1-acid glycoprotein (α-AGP) (orosomucoid) with an immunodiffusion method; (4) haptoglobin (Hp) with the method of Tarukoski,[23] and a rapid latex method; and (5) erythrocyte sedimentation rate (ESR) with a microhematocrit capillary tube, "mini-ESR."[15]

The white blood cell count (and differential) was performed in the routine laboratory, and the mini-ESR at the bedside, by the house staff. The remaining tests were carried out by one of us (J.R.H.).

The latex IgM and haptoglobin gave positive results at levels of 30 mg/100 ml or greater and 25 mg/100 ml or greater respectively (with a modification for haptoglobin). The latex C-reactive protein is positive when the concentration of C-reactive protein is approximately 0.8 mg/100 ml or above (normal values being less than 0.5 mg/100 ml in adults and less than 0.3 mg/100 ml in newborn infants[24]). The ratio of immature (band) forms to total neutrophils was assessed retrospectively. A ratio of 0.2 or greater was considered abnormal. The results of most tests were either not available or not calculated and, thus, did not influence decisions about antibiotic therapy.

Designation of infection status had to be made retrospectively, since all babies were considered at risk for, or demonstrated clinical evidence of, sepsis. Those babies whose blood (and sometimes CSF) cultures were positive within 48 hours were considered "proven." One case of meningitis with diagnostic CSF cells and glucose, but without a positive culture, was included because intravenous antibiotics had been started prior to cultures. Babies who received antibiotics for three days or less (and survived) were considered to be "not infected." Antibiotics were not given to 48 babies in this last group, probably on the basis of a "normal" WBC count.

The remainder may be considered uncertain, because antibiotics were given for longer than three days, although in many cases it was doubtful why antibiotics were continued. One may consider all these as "noninfected," but in several cases there was strong presumptive evidence of systemic infection (see "Results"). Values for sensitivity, specificity and predictive accuracy were calculated based on definitions by Feinstein.[25] Calculations of probability based on Bayes theorem were derived by using the work of McNeil et al[26] and confirmed with the nomogram of Fagan.[27]

RESULTS

Of 1,400 admissions, 376 babies had a "sepsis work-up" in the first week after birth which always included a blood culture and usually included urine and cerebrospinal fluid cultures. This number was comprised of 222 male and 154 female infants. Low-birth-weight infants (<2,500 gm) accounted for 219 of the total 376. There were 30 cases of proven infection, with 16 male and 14 female infants, of whom 18 were low birth weight. Blood cultures were positive in 29 and eight had positive CSF cultures, but no urine cultures were positive. Preliminary results with an original scoring system have been presented in abstract form.[28]

Table 1 shows five items that can be rapidly and easily evaluated and their relative value for predicting neonatal sepsis. It is apparent that individual tests are less predictive than a combination of tests. Consequently, babies were evaluated on the basis of 0 to 1 abnormality (negative "sepsis screen"), or 2 to 5 abnormalities (positive "sepsis screen").

Of the proven cases of bacterial sepsis, 28 had two or more tests positive. Details are provided in Table 2. The two "failures" were infants who grew *Escherichia coli* on blood culture taken shortly before death. In 20 babies, there was strong circumstantial evidence to indicate systemic infection and they may be considered "very probable." Details

TABLE 1. Results of Individual Tests (Compared to Two or More Positive) in 30 Proven Cases of Neonatal Sepsis from "At-Risk" Population of 376 Babies in First Week of Life

Test	Total Positive Tests	Positive Test with Proven Sepsis	Sensitivity (%)	Specificity (%)	Positive Predictive Accuracy (%)
Band/neutrophils ≥0.2	103	27	90	78	26
WBC <5,000/cu mm	37	15	50	94	40
CRP positive	64	14	47	86	22
Hp positive	28	9	30	95	32
"Mini"-ESR ≥15/1st hr	21	9	30	97	43
Any 2 or more positive	71	28	93	88	39

TABLE 2. Distribution of Positive Tests in Babies with Documented Infection

Case	Age at SWU*	Birth Weight (gm)	Sex	Positive Culture	Causative Organism	Survived or Died	Positive Tests				
							ESR ≥15	WBC <5,000/cu mm	Latex CRP Positive	Band/Neutrophils Ratio ≥0.2	Latex Hp Positive
1	3 days	2,637	F	Blood	GBS	S	···	4,300	+	0.50	−
2	1 days	1,984	M	Blood	*Haemophilus influenzae*	S	···	···	+	0.34	−
3	2 days	2,140	M	Blood	*Escherichia coli*	S	···	4,100	−	0.22	−
4	3 days	3,126	F	Blood/CSF	*E coli*	S	···	4,700	+	···	−
5	1 hr	2,910	M	Blood	Pneumococcus	S	···	···	+	0.34	−
6	4 hr	2,240	M	Blood	*Bacillus subtilis*	S	···	···	−	0.61	+
7	6 days	1,000	F	Blood/CSF	*B subtilis*	D	···	···	−	0.44	+
8	3 days	3,010	M	Blood/CSF	*E coli*	S	25	2,200	+	0.46	−
9	2 days	2,849	M	Blood/CSF	GBS	S	27	···	+	0.65	−
10	1 days	1,162	M	Blood	*E coli*	D	···	2,800	+	0.92	−
11	3 hr	1,340	F	Blood	GBS	D	···	4,600	−	0.31	−
12	3 days	1,520	M	Blood	*E coli*	D	···	···	−	···	−
13	2 days	900	F	Blood	*E coli*	D	···	···	−	0.23	−
14	4 hr	3,700	M	···	Meningitis (? *E coli*)	S	19	···	+	0.51	+
15	4 days	1,180	M	Blood	GBS	S	20	1,600	+	···	+
16	1 days	2,665	M	Blood	GBS	D	···	1,500	−	1.0‡	−
17	7 days	2,098	F	Blood	*E coli*	S	33	3,200	+	0.47	+
18	2 days	1,740	F	Blood	GBS	D	···	1,800	−	1.0‡	−
19	3 days	2,000	M	Blood	*E coli*	S	25	3,700	+	0.24	+
20	6 days	2,420	F	Blood/CSF	*E coli*	S	···	···	+	0.44	+
21	14 hr	1,400	F	Blood	GBS	D	···	1,400	−	1.0‡	−
22	2 days	2,948	M	Blood	GBS	S	···	2,800	−	0.86	−
23	6 days	2,900	F	Blood/CSF	*E coli*	S	32	···	−	0.21	+
24	6 hr	2,360	F	Blood	GBS	D	···	3,400	−	0.58	−
25	5 days	1,550	F	Blood/CSF	GBS†	S	···	···	+	0.25	−
26	5 days	3,000	M	Blood/CSF	GBS	S	22	···	−	0.30	−
27	7 days	5,960	M	Blood	*E coli*†	S	15	···	−	0.32	−
28	1 hr	1,210	F	Blood	*E coli*	S	···	···	−	0.52	+
29	1 hr	2,960	F	Blood	GBS	S	···	···	+	0.48	−
30	3 days	1,810	M	Blood	*Staphylococcus* (Coagulant positive)	S	···	2,800	−	0.37	−

* Abbreviations used are: SWU, sepsis work-up; CRP, C-reactive protein, Hp, haptoglobin; GBS, group B β-hemolytic streptococcus.
† Negative cultures and tests shortly after birth.
‡ One to two bands, no polymorphonuclear leukocytes.

are provided in Table 3. Of the 20, 16 (80%) would have been identified with the five-test scoring system. Six babies had proven viral infection. Table 4 shows the infection status in babies with a positive or negative "sepsis screen." Of the 320 babies without evidence of infection only 24 (8%) had two or more tests positive.

If only proven cases of sepsis are taken as positive and all others are regarded as negative, sensitivity of the "sepsis screen" is 93%, specificity is 88%, and the positive predictive accuracy is 39%. When the prevalence of proven sepsis is considered (30/376 or 8%), the random chance of predicting sepsis would be about 8% (compared to 39%).

If the cases considered as "very probable" (Table 3) are also considered positive, sensitivity, specificity, and positive predictive accuracy would be 88%, 92%, and 62%, respectively.

By using Bayes theorem (which takes the prevalance rate into consideration), and taking all non-proven cases as negative, the probability of sepsis with two or more tests positive is 40%, while the probability that sepsis is *not* present with *less* than two tests positive is more than 99%.

WBC count and differential alone would have identified 13 of the 30 proven cases. There were only four other babies with leukocyte count of less than 5,000/cu mm *and* a band/total neutrophil ratio of greater than 0.2 (ie, 76% of babies with both these findings had proven sepsis). Other combina-

TABLE 3. Details of Babies with Strong Evidence of Infection ("Very Probable" Infection)

Identification No.	Age at SWU*	Birth Weight (gm)	Sex	Factors Suggesting Infection
1/16†	2 days	3,544	M	Pneumonia
1/41†	8 hr	2,041	M	Maternal GBS, pneumonia
1/98†	2 hr	1,956	M	Petechiae and purpura; *Staphylococcus aureus* coagulant positive in CSF at 3 days (broth only)‡
1/104†	12 hr	2,840	M	Lethargy; tracheal aspirate, GBS
1/112	2 days	4,111	M	Fever; pustule grew *S aureus* coagulant positive
1/166†	4 days	2,280	M	Poor feeding; shock; response to antibiotics
2/33†	2 hr	1,300	M	PROM; maternal infection and antibiotics; lethargy
2/40†	2 days	2,060	F	Pneumonia; tracheal aspirate, GBS
2/46†	4 hr	2,980	F	Apnea; shock; double dose antibiotics IV before transfer and before blood culture
2/99†	6 hr	3,232	F	Fever; maternal GBS
2/114	4 hr	4,000	M	Maternal fever and antibiotics; tracheal aspirate, *Escherichia coli*; antibiotics IV before transfer and before blood culture
2/120†	3 days	2,395	F	Pneumonia; fever
3/17	1 hr	1,630	M	PROM 48 hr; pneumonia
3/32	1 hr	1,970	F	PROM; pneumonia
3/41†	2 hr	2,250	F	PROM 48 hr; pneumonia; Gram positive rod at 10 days (blood culture)‡
3/62†	1 hr	1,210	M	PROM 2 weeks; Apgar scores $1^1 \rightarrow 1^5$
3/67†	1 hr	1,480	F	PROM 4 weeks; foul smelling amniotic fluid; maternal fever and antibiotic therapy; pneumonia
3/69†	4 hr	3,440	F	Apnea; pneumonia with pleural effusion
3/71†	2 days	3,060	M	Apnea; lethargy; pneumonia
3/80†	2 hr	2,180	M	PROM; pneumonia

* Abbreviations used are: SWU, sepsis work-up; GBS, group B β-hemolytic streptococcus; PROM, prolonged rupture of membranes.
† Two or more tests positive.
‡ Considered to be contaminant organism.

TABLE 4. Infection Status of 376 Babies Investigated for Clinical Reasons during First Week after Birth According to Results of "Sepsis Screen"

Result of Sepsis Screen	Infection Status
71 positive	28 proven bacterial
	3 proven viral
	16 very probable
	24 not infected
305 negative	2 proven bacterial
	3 proven viral
	4 very probable
	296 not infected

tions were either less sensitive or less predictive. (Details of specific combinations are available from A.G.S.P.)

Gastric aspirate smears, platelet counts and IgM proved to be of little value in making an early diagnosis of bacterial sepsis. The value of α_1-acid glycoprotein is presently limited by the delay in obtaining results with an immunodiffusion technique (approximately 24 hours).

Gastric aspirate smears were performed in 101 babies where membranes had been ruptured for over 24 hours; 20 were positive, four of which (20%) were in babies with proven sepsis. Of the remaining 81 babies, six had proven sepsis within 48 hours without a positive smear.

Only 15 babies had low platelet counts (<150,000/cu mm) and only two had very low platelet counts (<50,000/cu mm), when the sepsis work-up was performed. There were two cases of proven sepsis with the former and one case with the latter.

A positive latex IgM, or IgM (by immunodiffusion) of 30 mg/100 ml or greater, was present in 45 babies, only three of whom proved to have bacterial sepsis. There were 68 babies whose α_1-acid glycoprotein level was greater than 50 mg/100, with 13 of the proven cases of sepsis included in this group. Only 23 babies had an α_1-acid glycoprotein level greater than 75 mg/100 ml and 8 (35%) had proven sepsis. The latex method for haptoglobin proved to be as effective as the Tarukoski method in identifying elevated levels.

DISCUSSION

The early diagnosis of neonatal sepsis is primarily based on clinical evaluation at the present time. Many babies are treated with several days of antibiotics because of *possible* infection, while waiting

for negative bacteriologic cultures—the "treat until cultures come back negative" approach. This study provides information on the value of several laboratory tests used in combination. Over 90% of infants with neonatal sepsis and 99% of noninfected infants were identified with five tests which can be performed within an hour. Such a sepsis screen is both simple and practical.

Although evaluation could undoubtedly be made more sensitive and specific if different values were used for different days of life, the values used have the virtue of simplicity and were applied during the first week of life. Manroe et al[17] showed that values of band/neutrophil ratio greater than 0.14 were associated with group B β-hemolytic streptococcus infection, but they documented values up to 0.17 on the first day of life.[29] Boyle et al,[30] have suggested that leukopenia of less than 10,000/cu mm is associated with infection on the first day of life. Similarly, erythrocyte sedimentation rate is usually only 1 to 2 mm for the first hour during the first 24 hours but may rise to 10 mm for the first hour in normal babies at the end of the first week.[15] The chosen criteria for the WBC count, band/neutrophil ratio and mini-ESR were all more stringent than the values quoted, thereby improving specificity. However, sensitivity was minimally sacrificed as a result.

It has recently been emphasized[31] that the "efficacy" of a diagnostic test is its ability to indicate the presence or absence of a disease. In this study, both sensitivity (if disease present, is test positive?) and specificity (if disease absent, is test negative?) showed a high index, 93% and 88%, respectively (Table 1). The clinician's questions are, "With a positive test, how likely is the disease to be present?" or "With a negative test, how likely is the disease to be absent?" The answer to the first question is provided in Table 1, where of 71 positive scores (two or more tests positive), 28 (39%) were associated with proven sepsis. Incidentally, another 16 (23%) had "very probable" systemic infection and three had proven viral infection. The answer to the second question is provided by the fact that there were 303 negative scores (0 to 1 positive) of 346 babies not proven to have bacterial infection, but only two of 30 babies with proven bacterial sepsis. The probability of sepsis being present depends not only upon the true and false positive rates but also upon the prevalence rate of sepsis in the population studied. Bayes theorem considers the prevalence rate and gives a 40% probability that sepsis is present if two or more tests are positive, and gives a 99% probability that sepsis is *not* present if the tests are negative.

Claims have been made for many individual tests as good indicators of neonatal sepsis. It is clear from this study that there is great variability in the value of the individual tests. Factors other than infection may produce positive tests (eg, fetal stress may increase band forms,[29] and inflammation produces an acute phase response). On the other hand, failure to mount an appropriate response may result in a poor prognosis.[32] The combination of simple tests described here requires no special laboratory equipment and could be readily available even in the smallest hospitals.

IMPLICATIONS

Of babies who had a sepsis work-up in the first week after birth, 8% had proven bacterial sepsis. Only 48 babies (13%) did not receive antibiotics. Thus, many babies are investigated for sepsis and receive antibiotics when they are not needed. In addition, no urine culture was positive in these babies, which supports the recent suggestion[33] that the risk of suprapubic bladder tap may not be justified in the first few days.

The greatest potential value of these tests is to exclude infection when uncertainty exists about the clinical condition of an infant (eg, when a baby is born after prolonged rupture of membranes, or has unexplained apnea or jaundice). These tests should also prove valuable in circumstances where antibiotic therapy has been given, either to the mother or to the infant, prior to arrival in the intensive care nursery. We have seen four or five of the tests positive in several babies admitted under such circumstances (among those babies included in the "very probable" category).

Earlier detection of the presence or absence of infection would save physician's time. A more rational approach to antibiotic therapy would decrease the number of babies started on antibiotics. Such a decrease could shorten the length of hospital stay in term infants and lessen the potential for emergence of resistant organisms.[34]

When uncertainty exists, these simple screening tests provide valuable adjunctive information in making decisions about antibiotics. However, when there is overwhelming clinical evidence of sepsis, it seems prudent to use antibiotics until cultures are negative.

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Decreased use of antibiotics using a neonatal sepsis screening technique

Antibiotic use was evaluated in an intensive care nursery before (1975-77, Group 1) and after (1978-80, Group 2) a "sepsis screen" was used at the bedside. All babies were evaluated prospectively, but the sepsis screen was available within an hour to influence decisions about antibiotics only in Group 2. The screen was positive if two or more of five simple tests were positive when investigation for possible sepsis was initiated. Group 1 and Group 2 had similar numbers of babies with proven sepsis and positive sepsis screens, and similar reasons for investigation, but there was a marked decrease ($P < 0.0001$) in antibiotic use in Group 2. When only those babies with a negative sepsis screen were evaluated, the result was even more striking. This study demonstrates that antibiotic use can be decreased with the help of simple, rapid, and inexpensive tests.

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THE EARLY SIGNS of neonatal sepsis may be "subtle, may be different at different gestational ages and are, unfortunately, common to various illnesses."[1] In most neonatal intensive care units, this difficulty in distinguishing the infected from the noninfected infant has resulted in the administration of antimicrobial drugs in a very high proportion of cases.[1]

Antibiotic usage is coming under increasing scrutiny, with efforts to improve accountability[2] and to decrease prophylactic administration.[3] In one adult intensive care unit, discontinuing "prophylactic antibiotics" decreased the cost attributable to antibiotics from 16.8 to 5% of the total annual pharmaceutical budget, without any increase in the incidence of septicemia.[4] In addition, there were fewer infections caused by gram-negative organisms.

Although antibiotic administration is relatively infrequent when all newborn infants are included,[5] the frequency of use is markedly increased in intensive care nurseries. Many more infants receive antibiotics than are proved to have infection by positive blood, urine, or cerebrospinal fluid cultures. In one review of neonatal antibiotic usage, as many as 28 babies were treated for every baby with documented sepsis.[6]

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Relier et al[7] have based their antibiotic usage upon a combination of risk factors (e.g., maternal fever during delivery), hematologic abnormalities (e.g., leukocytosis $> 30{,}000/mm^3$), elevated fibrinogen concentration, and cytobacteriologic examination of the placenta. They suggested that they had decreased "the potentially hazardous use of prophylactic antibiotics in an intensive care unit for distressed newborns" in Paris, although details of antibiotic usage were not provided.[7] A recent Scandinavian study also suggested (but did not show) that antibiotic usage in newborn infants may be decreased by improved diagnosis of sepsis.[8] An "acute phase reactants score" has been used in Japan to detect neonatal infection and was associated with a decrease in antibiotic use, but it is difficult to understand why, since results were not available for 24 hours or more.[9] We have recently described the results of a method for assisting in the early diagnosis of sepsis, using simple tests which are available within one hour.[10] This "sepsis screen" was used at the bedside from January, 1978, to facilitate decisions about antibiotics. The purpose of this report is to describe the resultant decrease in antibiotic usage.

MATERIAL AND METHODS

Infants admitted to the intensive care nursery of the Medical Center Hospital of Vermont were evaluated for neonatal sepsis in the first week after birth if certain risk factors or clinical manifestations were present. The prin-

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Table I. Composition of the two groups by sex, weight, gestational age, and day of evaluation for sepsis

	Group 1 (n = 284)	*Group 2 (n = 240)*	*Chi square*	P *value*
Males	173 (61%)	125 (52%)	4.14	< 0.05
Females	111 (39%)	115 (48%)		
Low birth weight (< 2,500 gm)	169 (60%)	167 (70%)	5.74	< 0.025
Preterm (< 37 wk)	167 (60%)	169 (70%)	7.63	< 0.01
Evaluated at				
Birth	147 (52%)	149 (62%)		
Day 1	68 (24%)	42 (18%)		
Day 2	29 (10%)	11 (5%)	(3) 8.45	< 0.05
Day 3-4	29 (10%)	21 (9%)		
Day 5-7	11 (4%)	17 (7%)		

Table II. Antibiotic usage in the two groups, showing the large increase in the percentage of infants not treated with antibiotics

	Group 1 (n = 284)	*Group 2 (n = 240)*	P *value*
Proven bacterial sepsis	22 (7.7%)	19 (7.9%)	ns
Survival with sepsis	14 (64.0%)	17 (89.0%)	ns
Proven viral infection	4 (1.4%)	3 (1.3%)	ns
"Very probable" infection	12 (4.2%)	22 (9.2%)	ns
Positive "sepsis screen"	47 (17.0%)	51 (21.0%)	ns
Antibiotics			
None	39 (14.0%)	113 (47.0%)	
1-3 days	134 (47.0%)	65 (27.0%)	
4-5 days	73 (26.0%)	16 (7.0%)	< 0.0001
> 5 days	38(13.0%)	46 (19.0%)	
Not treated with negative sepsis screen	38/237 (16.0%)	111/189 (59.0%)	< 0.0001

cipal risk factors were prolonged rupture of membranes (> 24 hours), evidence of maternal infection, and premature labor without adequate explanation. The principal clinical factors were lethargy, temperature instability, abdominal distension, unexplained apnea or cyanotic spells, and irritability (including convulsions).

Infants admitted between October, 1975, and December, 1977 (Group 1) were evaluated prospectively, but some results were not available immediately and the scoring system of the present "sepsis screen" had not been devised. Consequently, the assignment of scores to provide "negative" or "positive" sepsis screens was made retrospectively in Group 1, and decisions about antibiotics were not influenced by such assignment.

Those infants admitted between January, 1978, and February, 1980 (Group 2) were evaluated with the sepsis screen.[10] When two or more of five items were present, the screen was considered positive, whereas the presence of no or one item was designated negative. The five items were: (1) total leukocyte count less than 5,000/mm^3, (2) nonsegmented (immature) neutrophils divided by total neutrophils (brand/total neutrophil ratio) $\geq$ 0.2, (3) latex C-reactive protein positive, (4) latex haptoglobin positive, (5) mini-erythrocyte sedimentation rate $\geq$ 15 mm/first hour. Results of these tests were available at the bedside and entered into decisions to use or withhold antibiotic therapy.

The leukocyte and differential counts were performed in the routine laboratory using a Coulter counter and slide evaluation. The latex tests are slide agglutination tests with addition of a drop of reagent (antiserum) to a drop of serum with gentle agitation for four or five minutes, which can easily be performed at the bedside or under laboratory supervision. The erythrocyte sedimentation rate is performed using a microhematocrit tube, which is filled with blood, sealed at one end, and placed in a vertical position.

In both groups the decision to treat or not treat with antibiotics was made primarily by the house officer responsible for the baby. To some extent, such decisions

able III. Antibiotic use with the most frequent presenting features, showing a marked increase Group 2 in the number of babies *not* receiving antibiotics when those with proven bacterial viral infection were excluded

Risk/clinical factor	*Group 1 (n = 284)*		*Group 2 (n = 240)*	
	Number "not infected"	*Number not receiving antibiotics*	*Number "not infected"*	*Number not receiving antibiotics*
ROM alone	24	3 (13%)	40	30 (75%)
ROM and other	41	5 (12%)	40	18 (45%)
Maternal infection alone	11	2 (18%)	6	4 (67%)
Maternal infection and other	24	3 (13%)	20	5 (25%)
Premature labor alone	27	7 (26%)	43	30 (70%)
Premature labor and other	18	3 (17%)	21	14 (67%)
Lethargy alone	7	0 (0%)	6	4 (67%)
Lethargy and other	31	2 (7%)	18	8 (44%)
Temperature instability alone	4	0 (0%)	2	0 (0%)
Temperature instability and other	13	2 (15%)	8	2 (25%)
Apnea alone	16	3 (19%)	17	8 (47%)
Apnea and other	23	3 (13%)	16	4 (25%)
Cyanotic spells alone	21	1 (5%)	13	5 (38%)
Cyanotic spells and other	21	3 (15%)	8	3 (38%)

ROM = Premature rupture of the membranes.

may have been influenced by the attending staff. However, the attending staff was the same during the two time periods.

Infants with positive bacterial cultures of blood, cerebrospinal fluid, or urine were designated as having proven sepsis. Those with positive viral cultures were said to have a "viral infection," and some infants with pneumonia or other strong evidence of infection were called "very probable."[10] The rest were considered to be "not infected."

The populations studied in each time period (Groups 1 and 2) were evaluated for comparability by sex, birth weight, gestational age, day of investigation for sepsis, and reason for investigation of sepsis. Antibiotic use in both groups was also examined. Chi square analysis was used for statistical evaluation.

RESULTS

The composition of the two groups is shown in Table I. All babies who were evaluated for sepsis in the first week after delivery were included in this study. There were more males in Group 1, which might have predisposed to greater antibiotic use because males are known to be more susceptible to infection.[11] On the other hand, there were more low-birth weight and preterm infants in Group 2, which could have made greater antibiotic use more likely in this group.[12] There were some differences in the age at evaluation, but when babies evaluated at birth and within the next 24 hours were combined, these differences were barely significant.

The duration of antibiotic administration is shown in Table II. In Group 2 there was a highly significant ($P < 0.0001$) decrease in the number of babies receiving antibiotics, which was even more striking when only those babies with a negative sepsis screen were considered.

The incidence of proven bacterial sepsis was the same during the two time periods. Other minor differences were not statistically significant.

Three babies with proven infection and five babies with very probable infection were not detected by the sepsis screen. However, all these babies were treated with antibiotics. Details of two of the babies with proven infection have been reported.[10] The third was a baby with Group B beta hemolytic streptococcal infection on the first day of life who had an abnormal band/total neutrophil ration, but a leukocyte count of 8,000/mm^3.

To find out whether or not any unintentional bias had been introduced, an evaluation was made of the reasons for the investigation of sepsis in the two groups. Some minor differences were noted, but it was difficult to interpret whether these differences would bias antibiotic use in one direction or another. Therefore, the presenting features most commonly associated with sepsis were examined (Table III). In almost every category, the number of babies who did not receive antibiotics when the babies were "not infected" (proven sepsis or viral infection excluded) increased markedly in Group 2. Thus, one may conclude that although there were minor differences between the two groups, the decrease in antibiotic usage was not biased by such differences.

DISCUSSION

The usefulness of any diagnostic test (or group of tests) may be measured by the resulting changes in the treatment provided.[13] The purpose of the "sepsis screen" is to provide information which can be incorporated into decisions about antibiotic administration. If this screening technique is useful, a change in antibiotic usage would be anticipated.

Although the groups of babies evaluated in each time period were quite comparable, the number of babies who did not receive antibiotics increased markedly. In addition, of those babies in Group 2 who did receive antibiotics, there was a decrease in the number who were treated for four or five days, indicating that antibiotics were less likely to be continued when the cultures were reported as negative at 72 hours.

A small number of babies with either proven sepsis or very probable infection had negative sepsis screens at the time of initial evaluation. All of these babies were treated with antibiotics on clinical grounds. In other words, the sepsis screen was used to augment clinical evaluation; when the clinical evidence outweighed a negative screen, antibiotics were used.

In a recent survey by the Perinatal Section of the American Academy of Pediatrics,[14] over half of the 257 respondents used "prophylactic" antibiotics, and 60% were using antibiotics as often or more frequently than five years earlier. The most frequent reason for prophylactic treatment was prolonged rupture of membranes. In the present study, when prolonged rupture of membranes was one of the indications for evaluation of infection, the percentage of babies not treated with antibiotics rose from 13 to 60%, and the number untreated rose to 75% when the indication was prolonged rupture of membranes alone.

The results demonstrate a change in behavior of physicians making management decisions in the nursery. This change in behavior may be considered as the result of a change in attitude, based on knowledge of the value of the tests in predicting sepsis.[10] It is possible that the interest of the author (and enthusiasm for the tests) may have been transmitted to the housestaff, but the decisions about antibiotics were made primarily by the housestaff.

The ratio of babies treated with antibiotics to babies with proven sepsis fell from 11:1 to 6.6:1, in contrast to ratios of 15:1 and 28:1 in two Boston Hospitals.[6] Whether or not this ratio can be decreased further remains uncertain, but it may be feasible to use antibiotics for as short a time as possible. In a recent study of antibiotic usage, 12 of 36 courses of antibiotics in the newborn nursery were judged to have been too long.[15]

Excessive prophylactic use of antibiotics has been documented in several studies in adults and children.[4, 5, 15, 16] Because of the neonate's vulnerability to infection, most newborn infants who are suspected of having sepsis are given antibiotic therapy.[1, 14] This policy can result (and has resulted) in the emergence of resistant organisms which may be very difficult to treat.[1, 17] Despite the projected ability to produce new antibiotic substances in the future, "without judicious control of their application, the cycle of new and better drug-resistant organisms requiring new and better antibiotics will continue."[18]

Although the tests in this sepsis screening technique are not infallible, they are rapid and easily performed, and provide the basis for a more reasoned approach to antibiotic use. A significant decrease in the use of antibiotics may prevent the emergence of resistant organisms, decrease the chance of side-effects, and minimize costs. Current practices can be changed, given a simple, rapid, and inexpensive diagnostic technique.

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Detection of Neonatal Sepsis of Late Onset

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• Five tests have been used in combination to diagnose neonatal sepsis. This study describes their use in 56 infants evaluated between 8 and 60 days of age, who had nonspecific signs of infection at presentation, as well as further evidence in 524 infants evaluated in the first week after birth. When two or more of the five tests had abnormal results (leukocyte count <5,000/cu mm; immature/total neutrophils ≥0.2; ESR ≥15 mm/hr; latex C-reactive protein, positive; and latex haptoglobin, positive), a "sepsis screen" was considered positive. A positive screen was found in 23 infants, ten of whom had proved sepsis, and only two had no evidence of infection. With the addition of a leukocyte count greater than 20,000/cu mm, the remaining two cases of sepsis would have been detected. In those with a negative screen (n=33), 26 had no evidence of infection. The sepsis screen seems to be a useful adjunct in the diagnosis of neonatal sepsis during and beyond the first week.

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IN A previous report on 376 neonates evaluated in the first week after birth, several tests in combination provided a better prediction of sepsis than any test by itself.[1] These observations have been extended to more than 500 infants suspected of having infection during the first week of postnatal life. In addition, during the same period (1975 to 1980), similar observations were made in infants with clinical manifestations of sepsis who were evaluated during the next few weeks. This report will show that the "sepsis screen" is valuable beyond the first week.

Subjects and Methods

During the period October 1975 to March 1980, 524 neonates were evaluated for neonatal sepsis during the first week after birth in the Intensive Care Nursery of the Medical Center Hospital of Vermont, Burlington. During the same period, 56 neonates between the ages of 8 and 60 days were also evaluated for sepsis. In addition to a leukocyte count and differential cell count, mini-ESR, latex C-reactive protein (CRP), and latex haptoglobin tests were performed. When two or more of these five tests had positive results, the baby was considered to have a positive sepsis screen.[1,2]

The mini-ESR was performed by completely filling a microhematocrit tube, sealing it at one end, and placing it in a vertical position for one hour, as described by Adler and Denton.[3] An increased ESR is often determinable before the end of an hour. The latex CRP and haptoglobin are slide tests. Serum may be obtained by centrifuging a microhematocrit tube for five minutes. For the latex CRP test, one drop of reagent is added to one drop of serum, mixed, and agitated for four or five minutes. A positive test result is characterized by agglutination. For the latex haptoglobin test, incubation with antiserum for 15 minutes is required before addition of the latex reagent.

The infants in the early evaluation group (0 to 7 days of age) were a mixed group evaluated for risk factors (eg, prolonged rupture of membranes, unexplained preterm labor, maternal fever), clinical factors (eg, lethargy, apnea, temperature instability, abdominal distention), or a combination of the two.[2] The babies in the later assessment group (8 to 60 days of age) were evaluated purely on the basis of suggestive clinical features. Sepsis was considered proved when cultures of blood, CSF, or urine were positive for bacteria.

Definitions of sensitivity, specificity, and positive predictive accuracy are those provided by Feinstein.[4] Standard er-

Table 1.—Results of Sepsis Screen in Infants Aged Between 8 and 60 Days

Infection	Result of Sepsis Screen*	
	Positive	Negative
Proved sepsis	10	2
Proved viral infection	2	0
Necrotizing enterocolitis	2	0
"Very probable" infection	5	0
Superficial infection	2	5
No evidence of infection	2	26
Total	**23**	**33**

*Positive indicates two or more of five diagnostic tests positive; negative, less than two of five diagnostic tests positive.

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Table 2.—Laboratory Data at Initial Evaluation for Infants With Proved Sepsis

Patient No./Sex	Birth Weight, g	Gestation Age, wk	Age at Evaluation, Days	Reason for Sepsis Workup	WBC Count <5.0 (×10³/cu mm)	B/N* Ratio ≥0.2	Latex CRP	Latex Hp	Mini-ESR ≥15, mm/hr
1/M	3,410	40	20	Lethargy, apnea, hepatosplenomegaly	(32.9)	. . .	−	+	. . .
2/M	3,487	40	9	Fever, irritability, "dusky" spells	(27.2)	0.2	−	+	17
3/M	780	33	58	Hypothermia, poor perfusion	4.9	0.32	+	−	42
4/M	2,180	35	13	Lethargy	(24.9)	. . .	+	−	. . .
5/F†	690	33	13	Abdominal distention	. . .	0.4	+	−	. . .
6/M	2,268	40	21	Lethargy, poor perfusion	. . .	0.21	+	−	25
7/F	750	32	9	Abdominal distention, poor perfusion	3.8	. . .	+	−	. . .
8/F	3,390	38	33	Poor feeding	. . .	. . .	+	−	30
9/M†	4,280	40	18	Convulsion, poor perfusion	4.0	0.58	+	−	. . .
10/M	3,720	40	47	Lethargy, irritability, poor feeding	3.9	0.77	+	−	. . .
11/M	780	28	12	Apnea	. . .	. . .	−	+	25
12/M	2,700	37	13	Poor feeding, diarrhea, vomiting	. . .	0.49	+	−	20

*B/N ratio indicates immature/total neutrophils; CRP, C-reactive protein; Hp, haptoglobin.
†Died.

Table 3.—The Value of Individual Tests Compared With a Combination of Tests in 12 Proved Cases of Neonatal Sepsis From 56 Infants Evaluated Between 8 and 60 Days of Age

Test*	Total Positive Tests	Positive Tests With Proved Sepsis	Positive Predictive Accuracy, % (±SE)	Sensitivity,† % (±SE)	Specificity,‡ % (±SE)
WBC <5,000/ cu mm	7	4	57 (±7)	33 (±6)	90 (±4)
B/N ratio ≥0.2	17	7	41 (±7)	58 (±7)	79 (±5)
Latex CRP positive	22	9	41 (±7)	75 (±6)	71 (±6)
Latex Hp positive	10	3	30 (±6)	25 (±6)	86 (±5)
Mini-ESR ≥15 mm/hr	14	6	43 (±7)	50 (±7)	83 (±5)
Sepsis screen positive§ (any 2 or more)	23	10	43 (±7)	83 (±5)	74 (±6)
WBC ≥20,000/cu mm	6	3	50 (±7)	25 (±6)	88 (±4)
Modified sepsis screen positive	26	12	46 (±7)	100	69 (±6)

*B/N ratio indicates immature/total neutrophils; CRP, C-reactive protein; Hp, haptoglobin.
†Sensitivity: If disease present, test results positive.
‡Specificity: If disease absent, test results negative. Two cases of viral infection are excluded.
§Positive indicates two or more of the five diagnostic tests having positive results.

Table 4.—Value of Combinations of Tests in Detecting Neonatal Infection

Combination of Tests‡	Age 0-7 Days*			Age 8-60 Days†		
	Total Positive	Positive With Proved Sepsis	Positive Predictive Accuracy, %	Total Positive	Positive With Proved Sepsis	Positive Predictive Accuracy, %
WBC and B/N ratio	22	15	68	6 (+2)	3 (+1)	50 (50)
CRP and B/N ratio	50	15	30	10	6	60
Hp and B/N ratio	18	10	56	2	1	50
ESR and B/N ratio	21	9	43	7	4	57
WBC and CRP	14	9	64	6 (+2)	4 (+1)	67 (63)
ESR and CRP	19	8	42	8	4	50
Hp and CRP	22	8	36	2	0	0
Hp and ESR	9	6	67	3	2	67
WBC and ESR	5	4	80	3 (+1)	1 (+1)	33 (50)
WBC and Hp	5	3	60	0 (+5)	0 (+2)	. . . (40)
Any 2 or more	98	38	39	23	10	43

*Age 0 to 7 days: total tested, 524; proved sepsis, 41; sepsis incidence, 8%.
†Age 8 to 60 days: total tested, 56; proved sepsis, 12; sepsis incidence, 21%. Figures in parentheses indicate the addition of WBCs greater than 20,000/cu mm.
‡WBC indicates WBCs less than 5,000/cu mm; B/N ratio, immature/total neutrophils 0.2 or greater; CRP, latex C-reactive protein positive; Hp, latex haptoglobin positive; ESR, mini-ESR 15 mm/hr or greater.

Causative Organism	Source
Staphylococcus aureus coagulase positive	Blood
Streptococcus group A	Blood
Enterobacter	Blood
S aureus coagulase positive	Blood
Escherichia coli	Blood
S aureus coagulase negative	Blood, central venous catheter
S aureus coagulase negative	Blood
E coli	Blood, urine
Streptococcus group B	Blood, CSF
Haemophilus influenzae	Blood, CSF
Streptococcus group D	Urine
Pseudomonas aeruginosa	Blood

rors may be derived using the formula $SE=(pq/n)^{1/2}$ where p is the sensitivity or specificity and $q=1-p$, and the sample size is n. The 95% confidence limits are calculated as the percent ±2 SEs.[5]

Results

Of the 56 infants evaluated after 1 week of age, 45 were aged 8 to 30 days and 11 were aged 31 to 60 days. This latter group is not strictly "neonatal" by definition; but, almost without exception, these were preterm infants evaluated prior to or within a month of their expected date of delivery. The two groups will be considered together, as an 8- to 60-day age group.

Those infants evaluated for late-onset sepsis were classified according to the results of the sepsis screen (Table 1). Of those with a positive sepsis screen, there were five with very probable infection comprising 3 cases of pneumonia, 1 of enteritis, and 1 positive culture (*Escherichia coli*) of pleural fluid (from a chest tube). In two superficial infections, *Haemophilus influenzae* was cultured from an eye "wound" (cellulitis) and group B *Streptococcus* from periumbilical pustules. Two cases of necrotizing enterocolitis had pneumatosis intestinalis on abdominal films, and the viral infections were cytomegalovirus and herpes simplex infections, respectively. In those with a negative sepsis screen, the superficial infections were due to three *Staphylococcus aureus* coagulase positive (from eye drainage, ventriculoperitoneal shunt wound drainage, and a pustule) and two *Enterobacter* (from eye and wound drainage).

Details of the proved cases of bacterial sepsis are provided in Table 2. In this group there were two deaths (17%), nine males (75%), and four infants (33%) who were small (light) for gestational age. Details of the value of individual tests or the combination designated as a sepsis screen (any two or more of the five tests having positive results) are provided in Table 3. It should also be noted that the addition of an elevated total leukocyte count (>20,000/cu mm) in this age group would have detected two cases of infection due to *Sta aureus* (one of whom had osteomyelitis) that were "missed" with the original system. Only four other babies had a leukocyte count greater than 20,000/cu mm, one of whom also had sepsis. It is of interest that in the first week after birth, a leukocyte count greater than 30,000/cu mm was seen in only 17 infants (of 524). Only two had sepsis, but these were the only infants to have greatly elevated leukocyte counts (>30,000/cu mm) after the first two days (they occurred on days 6 and 7, respectively). Two other noninfected infants had counts greater than 20,000/cu mm after the first two days.

Although there are multiple combinations for two or more of the five tests, no specific sequence of testing seems to be superior. The value of specific pairs of tests are provided in Table 4, both for infants evaluated during the first week and for those between 8 and 60 days old. Some of the specific pairs are highly predictive when present, but detect considerably less than half of those with proved sepsis, whereas any two or more of the five tests (providing a positive sepsis screen) detect most cases of sepsis.

In Tables 5 and 6 it is suggestive that the more tests with positive results, the more likely is infection to be present. In both groups, few infants classified as "not infected" had a score of 2 or more.

Comment

Tests that are infallible in detecting neonatal sepsis remain elusive.[6] Despite our best efforts, it is frequently difficult to be sure if infection is present at the time of initial evaluation. This is primarily because neonatal sepsis masquerades under a wide variety of clinical manifestations.[7-9] Nevertheless, every effort should be made to make an early diagnosis, in order to provide effective treatment and to avoid using antibiotics indiscriminately.[2] During the first week after birth, a combination of tests was shown to be more reliable than any individual test.[1] The results presented in this article on older neonates confirm this impression. The sepsis screen (positive results of two or more tests) seems to be a valuable adjunct for infants between the age of 8 and 60 days. With a modification to include a leukocyte count greater than 20,000/cu mm (values greater than 16,000/cu mm are probably abnormal at this age)[10] as well as leukopenia (<5,000/cu mm), all those with sepsis would have been detected, with a positive accuracy of 46%. If other probable cases of systemic infection are included, the yield is even higher.

Considerable information can be obtained by careful assessment of the leukocyte and differential counts, and the mini-ESR is remarkably simple and easy to do. While waiting for results of these tests, one can perform the slide tests. The latex CRP is simple and can be done in ten minutes. In practice, it may be more economical to reserve the latex haptoglobin test until the other results are available. If none is positive, there is no practical advantage in proceeding with the latex haptoglobin test (the same is probably true when two or more of the others are positive). Results should be available within an hour.

In contrast to findings for infants in their first postnatal week,[1] an increased immature to total neutrophil ratio (≥0.2) was considerably less sensitive (58% vs 90%) in infants investigated between 8 and 60 days of age. On the other hand, a positive latex CRP had a sensitivity of 75% (vs 47% in the first week). This suggests that different tests are better at different ages and emphasizes the importance of using more than one indicator of infection. The frequency of increased levels of CRP in infants with infection supports the

Table 5.—Sepsis Screen Scores for Infants Aged Between 8 and 60 Days

	Score of Sepsis Screen,* No. (%)				
	0-1	2	3	4	5
Sepsis	2 (6)	4 (29)	5 (71)	1 (50)	0
Viral infection	0	2 (14)	0	0	0
Very probable infection†	0	5 (36)	2 (29)	0	0
Superficial infection	5 (15)	2 (14)	0	0	0
Not infected	26 (79)	1 (7)	0	1 (50)	0
Total	**33**	**14**	**7**	**2**	**0**

*The score is derived from the presence of five diagnostic findings (WBC count, less than 5,000/cu mm; immature/total neutrophils, greater than or equal to 0.2; ESR, greater than or equal to 15 mm/hr; latex C-reactive protein, positive; and latex haptoglobin, positive).

†Includes two cases of necrotizing enterocolitis.

Table 6.—Sepsis Screen Scores for Infants Evaluated in the First Week

	Score of Sepsis Screen,* No. (%)				
	0-1	2	3	4	5
Sepsis	3 (1)	24 (34)	8 (39)	4 (67)	2 (100)
Viral infection	3 (1)	3 (4)	1 (5)	0	0
Very probable infection	5 (1)	19 (28)	9 (43)	1 (17)	0
Not infected	415 (97)	23 (33)	3 (14)	1 (17)	0
Total	**426**	**69**	**21**	**6**	**2**

*The score is derived from the presence of five diagnostic findings (see Table 5).

contention of Sabel and Wadsworth[11] that this test is very valuable in infants during the first 30 postnatal days.

The finding that four (33%) of those infants with neonatal sepsis were small (light) for gestational age may be coincidental. However, it is interesting to note that a lack of T lymphocytes has recently been observed in infants who were small for gestational age, both at birth and during the first months after birth.[12] This finding could explain an increased susceptibility to infection.

Although the values for sensitivity and specificity are provided, the more clinically useful index is positive predictive accuracy.[4] Positive accuracy denotes how often the test was correct when its result was positive. With the comparatively high incidence of sepsis in the older infants evaluated, the tests used are more confirmatory than screening procedures. It should be noted that the babies included in this series were all being treated in an intensive care nursery. To date, this method of evaluation has not been applied in an ambulatory setting. However, the wide variety of manifestations suggesting sepsis (eg, poor feeding, lethargy, irritability, fever) and the low cost of the tests makes this a potential screening technique, in both the nursery and the emergency room.

The evidence to date suggests that this combination of tests (which are simple, rapid, and inexpensive) will prove helpful in making an early diagnosis of sepsis throughout the neonatal period, but more experience is required. It should be emphasized that a negative sepsis screen does not exclude sepsis (or other bacterial infection). Clinical evidence of deterioration should not preclude the use of antibiotics in the face of negative test results.

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Neonatal Sepsis Resulting from Possible Amniotic Fluid Infection

Risk and Detection

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Antibiotics are frequently prescribed "prophylactically" when the neonate is considered to be at risk for infection. The risk factors of prolonged rupture of membranes (>24 hours), maternal fever/infection, and/or unexplained preterm labor (suggesting possible amniotic fluid infection) were investigated in 276 babies. Only two of 150 babies investigated for a single factor proved to have sepsis, while of the 126 babies who had multiple factors, 13 had sepsis.

Several laboratory tests, used singly or in combination, were more helpful than clinical manifestations in predicting which babies were likely to be infected. Neonatal sepsis was present in 6 per cent of the total, in 10 per cent of neonates with clinical signs, in 21 per cent with an increased immature/total neutrophil ratio (I/T ratio $\geqslant$ 0.2), and in 36 per cent of infants with a positive "sepsis screen." The incidence of "infection" (sepsis and "very probable" infection) was 12 per cent overall, but was 14 per cent with neonatal signs, 44 per cent with I/T ratio $\geqslant$0.2, and 74 per cent in those with a positive sepsis screen. A leukocyte count < 5,000/cu mm and/or an I/T ratio $\geqslant$ 0.2 was 100 per cent sensitive for sepsis, but the sepsis screen was most "efficient" at detecting "infection."

Starting antibiotics on the basis of risk factors alone does not seem appropriate. In situations where amniotic fluid infection is possible, evaluation with the leukocyte count and differential (with or without other tests) could decrease the indiscriminate use of antibiotics, particularly when a single risk factor is the reason for suspecting infection.

IN RECENT YEARS, there has been an increasing conviction that amniotic fluid infections may *cause* rupture of the fetal membranes, rather than be the result.[1,2] In addition, premature rupture of the membranes occurs without demonstrable cause in approximately 50 per cent of cases,[3] and it is possible that many cases of preterm labor are the result of intrauterine infection.[3–5] Exposure to intrauterine infection (*i.e.*, infected amniotic fluid) clearly places the neonate at risk for developing infection.[6,7] In most centers, at the present time, babies delivered following prolonged and/or premature (preterm) rupture of the membranes are considered at high risk for the development of infection and are usually treated with antibiotics after taking specimens for culture.[8] Other categories of risk are treated in the same way.

This report assesses the value of using risk factors to suggest possible amniotic fluid infection to predict neonatal infection and contrasts

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this with the predictive value of several simple laboratory tests.

Subjects and Methods

The infants evaluated were part of a larger study in which all babies evaluated for neonatal sepsis in the first week of life were included.[9,10] Babies were delivered at the Medical Center Hospital of Vermont (MCHV) or transferred to the Intensive Care Nursery within the first 24 hours after delivery, during the period October, 1975 to March, 1980. The infants considered "at risk" for infection were those born following prolonged rupture of the membranes (more than 24 hours), unexplained preterm labor, maternal fever, and other evidence of maternal or fetal infection. Some of these infants also displayed clinical manifestations consistent with a diagnosis of infection (*e.g.*, lethargy, temperature instability, abdominal distension, apnea, etc.). All infants were evaluated with blood cultures, and many had cerebrospinal fluid and urine cultures. In addition, blood was sent for leukocyte count and differential, "mini-ESR,"[11] latex C-reactive protein, and latex haptoglobin tests. Results were incorporated into a "sepsis screen."[9]

Babies whose blood, CSF, or urine cultures were positive (usually within 48 hours) were considered to have "proven sepsis." Those with strong presumptive evidence of systemic infection (examples have been published previously[9]) were called "very probable infection," and the remainder were considered to be "not infected."

Values for sensitivity, specificity, predictive value and efficiency were calculated based on definitions by Galen and Gambino.[12] Sensitivity is derived from true positives/confirmed positive cases, specificity from true negatives/confirmed negative cases, positive predictive value (accuracy) from true positives/total positive tests, and efficiency from true positives plus true negatives divided by total evaluated. The data were gathered prospectively, but analyzed retrospectively. Statistical evaluation was performed using Chi-square analysis.

Results

Of 524 babies investigated during the first week of life, 296 were cultured within 6 hours of delivery and 110 during the subsequent 24 hours. Of these babies with an early evaluation, 276 were investigated because of prolonged rupture of membranes (>24 hours), and/or maternal fever (or other evidence of maternal infection), and/or onset of preterm labor without explanation. The yield of neonatal infection under such circumstances is documented in Table 1. During the study period there were 8,835 live births at MCHV, giving an incidence of very early sepsis of approximately 0.15 per cent, when outborn babies were excluded. Of particular note is the fact that only two babies out of 150 babies investigated for a *single* "risk" factor proved to have sepsis. The likelihood that sepsis was present (predictive accuracy) was considerably increased when these factors were combined or taken with other risk or clinical factors. The difference in the incidence of sepsis between those with a single risk factor (2/150) and those with multiple factors (13/126) was statistically significant ($\chi^2 = 10.93$, $p < 0.001$).

In addition, there were 23 babies evaluated for foul-smelling amniotic fluid, none of whom were infected; 20 babies with unexplained meconium-stained amniotic fluid (with one infected); and four babies with fetal tachycardia (with one infected).

TABLE 1. *Incidence of Neonatal Infection After Delivery Complicated by Certain "Risk" Factors in 276 Babies Investigated Within 30 Hours of Birth*

Risk Factors*	Total	Sepsis	Very Probable	Positive Predictive Accuracy	
				Sepsis	Infection†
PROM alone	61	0	4	0%	7%
MF alone	16	0	0	0%	0%
PL alone	73	2	1	3%	4%
PROM and MF	25	2	1	8%	12%
PROM and PL	11	2	1	18%	27%
MF and PL	1	0	1	0%	100%
PROM, MF and PL	1	0	1	0%	100%
PROM and other	42	4	6	10%	24%
MF and other	19	2	5	10%	37%
PL and other	27	3	1	11%	15%

* PROM = prolonged rupture of membranes, MF = maternal fever/infection, PL = preterm labor, other = usually "clinical factors."

† Infection = proven or very probable sepsis.

TABLE 2. *Detection of Infection in 276 Babies Evaluated Within 30 Hours of Delivery for Several "Risk" Factors*

Reason for "Sepsis Work-up"	Total Evaluated	Proven Sepsis	Infection "Very Probable"	"Sepsis Screen" Positive*	Proven Sepsis with "Sepsis Screen" Positive
Prolonged rupture of membranes	140	8	14	27	8
Maternal fever/infection	62	4	8	14	4
Preterm labor	113	7	4	17	6

* Positive = Two or more of five diagnostic tests positive (WBC < 5000 cu mm, I/T Ratio ⩾ 0.2, ESR ⩾ 15 mm/h, Latex CRP positive and Latex Hp positive).

Diagnostic Tests

The sepsis screen is positive when any two or more of the five tests utilized are positive. The yield from specific reasons for evaluation is provided in Table 2. The chance of predicting sepsis correctly before the tests are performed is 6 per cent for each of the major reasons for investigation. When the sepsis screen is used, the predictive accuracy improves to 29–35 per cent (an improvement of 5 or 6 times). When "very probable" infection is included, the incidence of infection is 10 to 19 per cent; but after using the sepsis screen, the correct prediction of infection increased to 59–79 per cent.

Some details of the sensitivity, specificity, and positive predictive accuracy of the tests used have been provided in a previous report.[9] Further details for babies investigated because of possible amniotic fluid infection are provided in Table 3. In this subgroup, the immature/total neutrophil ratio is as sensitive as the sepsis screen, although the positive predictive value is less.

The one case of proven sepsis missed with the sepsis screen had a total leukocyte count of 11.3 $\times 10^3$/cu mm with 2 per cent segmented and 1 per cent band-form neutrophils. The profound neutropenia was suggestive of infection and the baby was treated with antibiotics. The case missed by I/T ratio alone had a leukocyte count of 4.6 $\times 10^3$/cu mm and a positive latex CRP.

Of the 18 infants considered to have "very

TABLE 3. *Frequency, Predictive Value and Efficiency of Several Diagnostic Tests Used to Detect Neonatal Infection*

	WBC < 5.0 $\times 10^3$/cu mm	I/T Ratio ⩾ 0.2	WBC < 5.0 and/or I/T Ratio ⩾ 0.2	WBC < 5.0 and I/T Ratio ⩾ 0.2	Latex CRP +ve	Latex Hp +ve	ESR ⩾ 15 mm/h	Sepsis Screen +ve
Total (n = 276)	20	66	76	10	39	13	8	39
Proven Sepsis (n = 15)	7	14	15	6	6	4	1	14
Very Probable (n = 18)	4	15	16	3	13	1	5	15
Sensitivity (%)								
sepsis	47	93	100	40	40	27	7	93
infection*	33	88	94	27	58	15	18	88
Specificity (%)								
sepsis†	95	80	77	96	87	97	97	90
infection*	96	85	81	99	92	97	99	96
Positive Predictive Accuracy (%)								
sepsis	35	21	20	60	15	31	13	36
infection*	55	44	41	90	49	38	75	74
Efficiency (%)								
sepsis	92	81	82	95	85	93	92	91
infection*	89	85	87	91	88	87	89	95

I/T = Immature/Total Neutrophils; CRP = C-reactive protein; Hp = Haptoglobin.

* Proven sepsis and "very probable" infection.
† "Very probable" included as not infected.

probable" infection, 15 had a positive sepsis screen, but 15 also had an I/T ratio ⩾ 0.2 (Table 3). The sensitivity of the other individual tests was poor at this age, especially ESR. If a level of ⩾10 mm/h was used, four more cases of sepsis and two of "very probable" infection would have been included. (This seems a reasonable modification, since normal levels are 0–2 mm/h in the first two days[11]). Sensitivity of 100 per cent can be attained for sepsis by combining a low leukocyte count with an elevated I/T ratio. Thus, in doubtful cases, it may be most helpful to use the I/T ratio as the "screen" and apply one of the other tests to "confirm" infection.

TABLE 4. *Effect of Birth Weight and Gestational Age upon Outcome in Infants "at Risk" for Infection*

Reason for Sepsis Work-up	Subgroup	Total	Proven Sepsis	Very Probable Infection	Died
PROM*	<2500 g	101	5	12	8 (3)†
	⩾2500 g	39	3	2	1
Preterm Labor	<2500 g	106	7	4	9 (3)
	⩾2500 g	7	0	0	0
Maternal Fever/ Infection	<2500 g	38	2	6	3 (1)
	⩾2500 g	24	2	2	2
PROM	<34 weeks	61	4	8	7 (2)
	⩾34 weeks	79	4	6	2 (1)

* Prolonged rupture of membranes.

† Figures in parentheses are the number of infants who died from sepsis.

Low Birth Weight and Prematurity

Infants of low birth weight (<2500 g) are generally held to be at greater risk for infection than those who are above 2500 grams. In our study, the number of babies with sepsis is too small to draw any definitive conclusions, but there was no obvious disadvantage (Table 4) in the incidence of sepsis for those of low birth weight or those born at an early gestational age. However, it should be noted that all the infants who *died* from sepsis (in these categories) were low birth weight.

Discussion

This study looked at features which might indicate amniotic fluid infection, focusing on the significance of prolonged rupture of membranes, unexplained preterm labor and maternal fever (either singly or in combination). There is evidence to support the idea that amniotic fluid infection may *cause* rupture of membranes,[1,2] and that it is an important cause of preterm labor.[3,5] Maternal fever is usually considered to be an indicator of chorioamnionitis,[6,13,14] but it may be a late sign.[1]

The relationship between chorioamnionitis and prematurity, and the importance of "ascending infection" were stressed by Benirschke more than 20 years ago.[15] Blanc reported that when membranes were ruptured for more than 24 hours, 74 per cent of amniotic fluids obtained transabdominally had positive cultures.[16] There was a strong association between chorioamnionitis (determined histologically) and congenital pneumonia, but overt neonatal infection following exposure to amniotic fluid infection was rare (although *colonization* was usual).[16] In a recent study, 72 per cent of preterm infants were shown to come from an infected environment, but only 4 per cent had definite evidence of neonatal infection.[5]

The results of a recent questionnaire indicated that in the groups studied here (PROM, etc.) prophylactic antibiotics were given to the majority of infants.[8] Studies of prolonged and/or premature (preterm) rupture of the membranes have shown the incidence for proven infection to be between 0 per cent and 8 per cent.[15,17–20] The current study is consistent with these findings, with an incidence of 6 per cent for the most frequent risk factors, and suggests that if all of these babies are started on antibiotics, more than 90 per cent may be treated unnecessarily. With the use of a few simple diagnostic tests, it is possible to greatly improve predictive accuracy and, thereby, to decrease the use of antibiotics.[10]

Clinically, it is useful to know what the chances are that a positive laboratory test indicates infection (positive predictive value). In neonatal infection, the single most helpful and sensitive test seems to be the immature to total neutrophil ratio.[21–23] Higher ratios have been associated with depletion of neutrophil reserves in the bone marrow.[23] In this series, all the septic infants, but one, had I/T ratios greater than 0.30. The predictive value for sepsis of this test alone was 21 per cent, but the addition of other tests increased the predictive accuracy to 36 per

cent. Although the combination of a low leukocyte count *and* an elevated I/T ratio is relatively uncommon, it is highly predictive of infection (60% for sepsis and 90% for combined "infection"). A low leukocyte count *and/or* an elevated I/T ratio is most sensitive; but of the tests with high sensitivity, the sepsis screen is most efficient.

Although the yield of infected babies was low (2 of 150) when a single risk factor was used to make the diagnosis, the yield improved to 9 of 88 when clinical features were present in the baby. It is possible that a better correlation of sepsis with amniotic fluid infection might be achieved if more objective evidence of chorioamnionitis was used as the starting point (*e.g.*, culture of amniotic fluid obtained with an intrauterine catheter or by abdominal amniocentesis[24] or use of maternal C-reactive protein levels[25]).

The infant born at a gestational age of less than 34 weeks is considered to be at high risk for both pulmonary immaturity and developing infection. There is little agreement about the best approach to the infant with premature rupture of membranes,[6] but the initiation of a reasonably aggressive approach to such patients adopted at the Medical Center Hospital of Vermont[6] may account for the lack of difference in the incidence of infection above and below 34 weeks (Table 4).

Because of the association of infection with preterm labor, the importance of attempting to prevent infection of the amniotic fluid has recently been stressed.[5] In those babies who emerge from such an environment, it seems equally important to attempt to make a specific diagnosis of infection, to decrease the indiscriminate use of antibiotics.

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